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INHERENT FACTORS RELATED TO ABSORPTION OF MINERAL ELEMENTS BY PLANTS

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(WITH EIGHT FIGURES)

That different species of plants differ widely in their ability to grow on infertile soils has long been recognized by farmers. Rye and buckwheat are well known "poor land" crops, while in many localities scrub pine, sassafras, pennyroyal, broomsedge (*Andropogon virginicus*), and poverty grass (*Danthonia spicata*) are notoriously indicative of impoverished soils. They enjoy this reputation because of their ability to live and thrive where other plants would fail. On the other hand, corn, barley, wheat, and Kentucky bluegrass are generally regarded as "strong land" crops, and into this same general class presumably fall many of the oaks and hickories, the black walnut, and the tall ambrosia (*Ambrosia trifida*).

During recent years much interest has arisen in connection with the manner in which plants absorb mineral elements, the precise nature of the processes involved, and the underlying causes for the differences manifested by different species with respect to the absorption of essential and non-essential elements. From the first investigators have recognized differences in character, size, and extent of

the root systems of plants. It is readily apparent that a plant with a relatively large and actively absorbing area will afford better opportunity for the intake of materials from the soil solution than one with a smaller absorbing area, but as yet we have no very satisfactory method for measuring total areas of root surface exposed, much less a method for determining the actively absorbing portions. In addition to root areas, such factors as concentration of CO_2 in and around the root surfaces, permeability of root cells, acidity of cell sap, and adsorptive and absorptive nature of root colloids need due consideration. In all of these we seem to be still without adequate knowledge of the activities and operation of the root hair. The work herein reported deals chiefly with two phases of the problem, namely, the effect of calcium supply on sap acidity and the intensity of carbon dioxide production by plant roots. Other possible factors receive minor consideration.

Historical

There is an extensive literature bearing upon the general problem of mineral absorption by plants. No attempt has been made to review this literature exhaustively. In the following pages only brief reference is made to the results of certain other workers which relate to root systems, carbon dioxide production, permeability, and the acidity of vegetable saps as influential factors in absorption phenomena.

Root systems and root areas

Considerable work has been done on the root systems of plants, but there is little accurate knowledge concerning the areas exposed within the soil. In the United States much of the earlier work was done by agricultural experiment station workers. Miller (**22,23**) has summarized this, together with certain foreign material, and added to it his own work on corn and the sorghums. More recently, Weaver and his co-workers have engaged in a systematic investigation of root systems of both agricultural and non-agricultural plants west of the Missouri River. Weaver, Jean, and Crist (**49**) report that plant roots coming in contact with a fertilized layer of soil retard downward growth, while branching and developing much more abundantly in the fertilized region. Weaver (**48**) states that

rye usually has a more branched root system than wheat or oats grown under similar conditions, thus partially explaining why rye will thrive on poorer and more sandy soils than other cereals. In a recent article, WEAVER, KRAMER, and REED (**50**) report determinations of "total absorbing area exclusive of root hairs" for Kanred wheat, as calculated from numerous measurements of lengths and average diameters. Ordinary laboratory determinations and estimates of the increase in area due to root hair formation, for various seedling plants germinated in moist air, indicate an increase of five to twenty or more times, but the number and development of root hairs normally occurring in soils is less certain, and presumably varies considerably, depending upon conditions. In this connection the early work of SCHWARZ (**33**), and later work of SNOW (**34**) and HESSE (**13**) are suggestive.

All investigations appear to support the statement that there may be wide variations in the comparative extent and development of the root systems of different species of plants.

CARBON DIOXIDE AND PLANT ROOTS

The solvent action of aqueous solutions of carbon dioxide upon soil minerals is too well known to justify discussion in this brief review. The important matter for consideration here is the established fact that plants give off considerable amounts of carbon dioxide from their roots during growth. Since the time of LEIBIG and SACHS it has been known that plant roots will leave their traces upon the surface of marble by etching. There are abundant researches upon the nature of root excretions. The work of CZAPEK (**8**) gave strong indication that carbonic acid is the only acid of importance normally excreted by the roots of higher plants. From a series of investigations, STOKLASA and ERNEST (**37,38**) decided that roots of common crop plants give off acids other than carbonic only when normal respiration is disturbed by insufficient oxygen supply. They refute CZAPEK's view that mono-potassium phosphate is excreted in significant quantity from root hairs, and believe that plants are able to absorb relatively insoluble forms of minerals in proportion to the output of CO_2. KOSSOVITCH (**17**), working with mustard in solution culture, concluded that CO_2 excretion might in

some cases be important in rendering soil minerals available. ABERSON (1) made electrometric determinations of the acidity of root excretions from young plants of oats, buckwheat, and others, and concluded that, although root excretions contained no noteworthy concentration of H-ion, still the H-ion concentration of a saturated solution of CO_2, when concentrated in the mucilaginous wall of the root hair, is probably sufficiently effective to dissolve certain soil minerals, particularly the phosphates. HALL (12) had earlier reached a similar conclusion, but from their experiments with crops grown in pots containing different phosphates, and treated with CO_2 gas, PFEIFFER and BLANCK (28) regard any amounts of CO_2 given off by the plant as insufficient to account for the phosphates taken up. NELLER (24) found that growing plants greatly increased the oxidation processes in a soil of average productiveness, as indicated by the CO_2 evolved, and suggests a symbiotic relationship between soil oxidizing organisms and growing green plants. TURPIN (47) determined relative amounts of CO_2 produced by soils growing oats and millet, as compared with uncropped soils. He concluded that the influence of the crop as a producer of CO_2 has been underemphasized, and that the CO_2 output from the plant is largely the result of root respiration, rather than decay of root particles. RUSSELL and APPLEYARD (30) found no significant differences in the CO_2 content of soil air in which different species of plants were growing, but in a later publication (31) they found a considerable increase in the production of CO_2 in the soil during the period of rapid growth of the crop, and another and larger increase at the time of ripening. They consider the latter increase to be inadequately explained as a respiration effect. BIZZELL and LYON (4) determined the period of greatest apparent production of CO_2 by oats to be at the time of bloom, and subsequent to blooming found a marked decrease. This they attribute to a depressing influence of the crop on bacterial action.

The numerous investigations in this field indicate that growing plants give off quantities of CO_2 sufficient in amount to be of importance in the solution of soil minerals in the region of the absorbing roots. The plant may also exert an appreciable effect on oxidation processes in the soil, but the nature of such an effect seems to be somewhat less certain.

Permeability and general absorptive phenomena

Many investigations dealing with general absorption phenomena have been made, but comparatively few direct experiments upon the passage of dissolved substances into root cells are available. It is well known that many factors affect the absorption of ions by plants, and also that cell membranes are capable of changes in permeability, depending upon the conditions to which they are subjected. Stiles (**36**) has recently published an exhaustive review of the investigations dealing with permeability in general. The work of Meurer (**21**), Pantanelli (**26**), and others showed that the anions and cations of a salt frequently enter the plant in dissimilar proportion. The work of Johnson (**15**) indicated further that the presence of dead cells may have an appreciable effect upon the relative absorption of the constituent ions. True (**40**,**41**) emphasizes the importance of calcium in rendering other elements physiologically available to the plant. Hoagland (**14**) stresses the influence of one ion on the absorption of another, and the importance of conditions of light, temperature, and humidity. Recently Comber (**7**) has introduced the idea of a single, intimate, continuous root hair-soil particle colloidal system through which the plant may actually absorb colloidal particles, and whereby the dissolution of soil particles is accomplished by the presence of organic compounds in the root hair so attached. Finally, Gericke (**10**) has reached the interesting conclusion that the temporary depletion of certain essential elements in the soil is an important beneficial factor in the production of maximum crop growth.

The evidence from this field of endeavor reveals a complexity of factors and conditions, which at present are not well understood and hence difficult to evaluate, but whose general importance in nutrition and growth presumably is very great.

Acidity of plant saps

As early as 1907 Lemmermann (**19**) compared the titration acidities of root extracts from a considerable number of different representatives of the grass and legume families. He found characteristic differences, and concluded that the greater acidities generally prevalent in the legumes must be an important factor in their superior ability to secure mineral elements from the soil.

During recent years much interest has been evidenced in the application of H-ion studies to biological problems. In this connection TRUOG and his co-workers have investigated the acidities of various plant saps as determined by the use of the hydrogen electrode. TRUOG and MEACHAM (**46**) found clover, alfalfa, soy beans, and buckwheat to have less acid saps when grown on limed than on unlimed soils. CLEVENGER (**6**), however, found the reverse to be true. He states, "with the exception of buckwheat, the tops of the limed plants were usually more acid." He found also that acidity varies somewhat with the stage of development of the plant, and that different portions, as leaves and stems, may yield different results. HAAS (**11**) showed that there may be an acidity gradient from tops to roots, sweet clover showing acid, neutral, and alkaline saps in the same individual. The range in this case was from P_H 5.82 in the top to P_H 8.00 in the root for a plant in its second year of growth, on soil of P_H 7.68. He showed an increase in acidity with age, and a decrease with a reduction of the normal illumination. BAUER (**2**) grew various plants under treatment with rock phosphate in sand cultures, and found no definite relation of the amount of plant growth to the extent of the root systems, the phosphorus content, or the acidity of the plant juices. BAUER and HAAS (**3**) studied the effects of limestone, leaching, phosphate salt, and nitrogen salt on the acidity of the soil medium and plant juices, each treatment showing considerable effect. They state that acidity was closely related to the growth and feeding powers of the plant. KAPPEN and ZAPFE (**16**) determined the acidity in roots and tops of beans and lupines at blossoming time, when growing on a sandy loam soil under treatment with 1, 5, and 10 per cent additions of calcium carbonate. Sap acidity was not appreciably affected by the additions of limestone, the conclusion being that although plants grown on limed soil probably take up more lime, nevertheless they are able to maintain the same degree of acidity in their juices as on unlimed soil. BRYAN (**5**) in general secured increased sap acidities with increased acidity of the culture media, but corn tops gave no significant change. Root juices followed culture media much more closely in reaction values than did leaf juices. NEWTON (**25**), working with barley, peas, and beans in solution cultures, concluded that the

H-ion concentration of the plant sap is not increased by limiting the plant's supply of calcium.

From the data available it would seem that the acidities of expressed saps commonly range from approximately P_H 7.00, for such plants as sweet clover and alfalfa, to about P_H 3.00 for rhubarb and red sorrel. Variations may occur in different portions of the same plant. The evidence as to the effect of external conditions and treatment of culture media on sap acidity is somewhat conflicting.

Materials and methods

EXPERIMENT I: SAP ACIDITY AND CALCIUM SUPPLY.—Since, according to TRUOG, one of the chief functions of calcium in the plant is to neutralize sap acids, it was thought desirable to seek additional light on the matter by studying the effect of calcium supply upon the acidity of the sap of tomato plants. Accordingly, a plan was devised whereby the rates of calcium supply would vary across three reaction values in the culture solution. This is shown in table I, where the numbers in parenthesis represent the numbers of the supply bottles. The plants were grown under greenhouse conditions, with a continuously renewed supply of solution.

TABLE I

VARIATIONS IN CULTURE SOLUTION

Calcium content	P_H 4	P_H 5	P_H 6
High, 1000 ppm.....	(1)	(4)	(7)
Medium, 100 ppm.....	(2)	(5)	(8)
Low, 10 ppm.....	(3)	(6)	(9)

EXPERIMENT II: CARBON DIOXIDE FROM ROOTS.—In an effort to determine, quantitatively, the production of CO_2 by the roots of representative species of plants, Reid's Yellow Dent corn, Oderbrucker barley, Japanese buckwheat, Rosen rye, Manchu soy beans, and Canada field peas were grown in soil in the greenhouse in jars which were continuously aspirated over a period of six weeks. Weekly determinations of the CO_2 given off by planted and unplanted jars were made. It was desired also to learn something of the size and extent of the root systems developed by the various kinds of

plants, and for this reason dry weight determinations and estimations of relative root areas were made. For purposes of comparison a supplementary trial was arranged, in which the plants also were grown in air-tight containers, in quartz cultures, for a similar period of time.

APPARATUS FOR EXPERIMENT I.—The tomato plants were grown in glazed earthenware jars of 2-gallon capacity, equipped with inlet

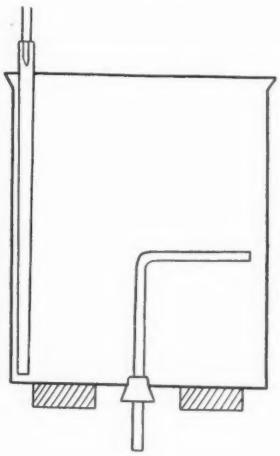

FIG. 1

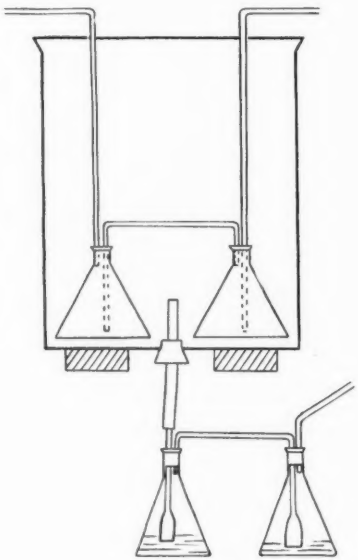

FIG. 2

FIGS. 1, 2.—FIG. 1, diagrammatic sketch of culture jar used for growth of tomato plants; fig. 2, diagrammatic sketch of soil culture jar with absorption flasks.

and outlet tubes as shown in fig. 1. The culture solution was allowed to enter at the bottom of the jar on one side, and was taken out at a point higher up on the opposite side. Into the upper end of the inlet tube was thrust a piece of smaller tubing drawn to a point. This gave a visible dripping point at which the rate of flow of solution could easily be seen at all times. The rate of flow was

adjusted by means of a screw clamp attached to a piece of extra heavy gum tubing connecting the dripping point with the supply system. The twenty-six jars so equipped were filled with pure quartz sand, and the roots of the growing plants bathed with a continuous flow of the solution described later. This was supplied from nine large glass bottles of 5-gallon capacity. To prevent algal growth the entire system, except for the dripping points and a narrow strip vertically along each bottle, was painted black. The bottles were calibrated in liters in the unpainted strip, thus allowing for additional check upon the flow of the solutions. In addition to the heavy coat of black paint, the supply bottles were doubly wrapped with heavy brown paper. The culture jars were supported 6 inches above the bench upon narrow boards placed a few inches apart, the outlet tubes projecting from the bottoms of the jars down into the opening between the boards. Glass bottles of convenient size were placed beneath the outlets when it was desired to collect and test the solutions coming from the jars.

CULTURE SOLUTION.—In the preparation of a suitable culture solution, it was originally intended to employ potassium hydrogen phthalate as a buffer material, but preliminary experiments showed that it exerts a toxic action on young tomato plants. For this reason it was discarded in favor of sodium carbonate, as described by BRYAN. Calcium was supplied at three different rates. The composition of the unadjusted solution is shown in table II.

The various solutions were made up in nine and eighteen liter quantities, and adjusted to the desired reaction by adding sodium carbonate in suitable quantity, as indicated by the electrometric titration curves. The actual amounts added varied with the reaction values, and to a less extent with the calcium content. Iron was supplied directly to the inlet tubes of the culture jars as ferric phosphate suspension.

SUPPLY OF CULTURE SOLUTION.—The rate of supply of culture solution varied somewhat, due chiefly to temperature changes, but by regulating the screw clamps four to six times daily the variations were not great. At night the flow was much less subject to change. The experiment was begun with about 600 cc. of solution passing through each pot in 24 hours. This was gradually increased

to almost 2 liters when the plants were at their largest. The object was to supply the solution in quantities sufficient to prevent radical changes in the reaction values of that contained within the jars, and that this was accomplished is shown by the data in table III. With some of the pots there was an intermittent siphoning effect, when a considerable portion of the solution would pass out at one time. Others dripped steadily with the inlet tubes.

TABLE II

UNADJUSTED CULTURE SOLUTION

Substance	Grams per liter	Parts of element per million parts of water
H_3PO_4	0.8055	255 of P
KNO_3	0.8321	321 of K 115 of N
$NaNO_3$	0.8440	139 of N 228 of Na
$MgSO_4$ 0.7 H_2O	1.2142	121 of Mg 160 of S
$CaCl_2$ High	2.7750	1000 of Ca 1770 of Cl
Medium	0.2775	100 of Ca 177 of Cl
Low	0.0277	10 of Ca 17 of Cl

METHOD OF GROWING PLANTS.—On January 16 seeds of the Bonny Best variety of tomato were sown in soil and the plants repotted from time to time during the next four months. During this period the most vigorous plants only were saved, one being chosen eventually as the stock plant from which the cuttings were made. Preliminary trial showed that cuttings could satisfactorily be rooted in tap water and subsequently transferred to sand or soil as desired. On July 14 the cuttings were set in the culture jars. At this time also thirteen of the twenty-six jars were planted to seeds of the STONE variety, and these later thinned to six plants per jar. Supply bottles nos. 2, 4, 6, and 8 supplied four culture jars each,

while nos. 1, 3, 5, 7, and 9 supplied only two jars each. In each case the number of jars growing cuttings and seedlings was the same, or a total of thirteen for each.

PREPARATION OF MATERIALS AND DETERMINATION OF REACTION VALUES.—In all determinations of reaction values the standard Leeds and Northrup Type K potentiometer was used, equipped with a bubbling hydrogen electrode of the Hildebrand type, and a saturated calomel electrode with liquid junctions across saturated KCl solution. Plant saps were secured by the usual method. After harvest the plants were immediatedly frozen solid, macerated in a meat chopper (or mortar where the quantity of material was small),

TABLE III

P_H RANGE OF CULTURE SOLUTION

SUPPLY BOTTLE	CALCIUM CONTENT (PPM)	AS MADE UP	ISSUING FROM CULTURE JARS	
			Cuttings	Seedlings
1	1000	4.01–4.05	4.80–4.87	4.93–5.05
2	100	4.07–4.10	5.34–5.40	5.25–5.37
3	10	4.27–4.28	5.15–5.30	5.00–5.33
4	1000	4.90–4.96	5.05–5.16	5.08–5.26
5	100	5.35–5.40	5.44–5.55	5.51–5.55
6	10	5.36–5.40	5.47–5.79	5.25–5.51
7	1000	5.35–5.45	5.19–5.30	5.21–5.35
8	100	5.96–6.05	5.77–6.00	6.03–6.18
9	10	6.03–6.12	5.96–6.15	5.97–6.22

folded in muslin cloths, and pressed in a hand press. The expressed juices were then centrifuged before the determination of reaction values. In a few cases where, due to the poor growth of the plants, the quantity of sap secured was quite small, it was diluted with an equal volume of redistilled water.

APPARATUS FOR EXPERIMENT II.—In this experiment also, the soil cultures were arranged in glazed earthenware jars of 2-gallon capacity with perforated bottoms, as already described. Each jar contained two auto-irrigator cones of porous clay, as described by LIVINGSTON (**20**) (fig. 2). These were connected in series within the jars and eight jars joined together, all supplied with distilled water from a single source, and all drawing their moisture against a single U-tube of mercury. At the lowest point of the bend the U-tube was

provided with a small outlet tube closed by a pinchcock. Through this, mercury was withdrawn from time to time as the plants increased in size and rate of transpiration. Except for a defective rubber connection between the cones in one of the unplanted jars, which was easily and quickly repaired, the entire system worked admirably. Small quantities of air accumulated in the tubes frequently, and were readily removed by drawing them forward through the system. Continuous aspiration over the six weeks' period was accomplished by the use of the large bottles described in the preceding experiment, and subsequently converted into aspirator bottles. The rate of flow was regulated by means of small-bore glass stopcocks attached at the lower end of the outlet tubes.

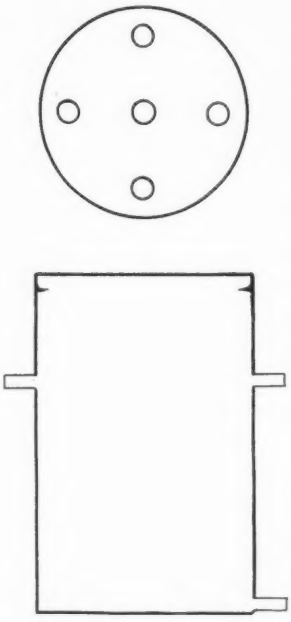

FIG. 3.—Galvanized iron tank used as air-tight container in experiment II.

In the supplementary trial the same kinds of plants were grown in galvanized iron containers of 2.5 gallons capacity, designed especially for the experiment as shown in fig. 3. The tanks were coated inside with paraffin, filled with pure quartz sand, the high calcium culture solution already described added, and the tops sealed off with a heavy layer of paraffin. In this holes were cut through which the plants were grown, and when they had attained sufficient size the openings around the stems were filled with a suitable mixture of plastic clay and vaseline. It was found that by leaving the holes slightly sunken and keeping the depressions filled with water, no particular difficulty was experienced in maintaining the containers air-tight. Without the covering of water the clay tended to dry out and crack with the expansion of the plant stems. The small amounts of water so held likewise gave excellent indication of the tightness

of the seals about the stems. Carbon dioxide-free air was drawn across the roots of the growing plants. Fresh culture solution and distilled water were added frequently. Aspiration and CO_2 determinations were similar to those described for the plants grown in soil.

ASPIRATION OF CULTURE JARS.—The aspirator bottles were accurately calibrated in liters, by which means the flow of air through the culture jars was kept practically constant. Nevertheless, constant care and attention were required, and even so there would sometimes be differences of as much as a liter in the amount of water dripping from the bottles during the night. Dissolved air in the tap water used was the greatest source of trouble. It was necessary to regulate the flow as frequently as five or six times daily in order to keep the bottles emptying together. When differences occurred they were always adjusted within a few hours, so that the total aspiration was the same for all jars within any given twelve-hour period. The rate of aspiration was increased slightly from week to week as the plants grew.

ABSORPTION AND DETERMINATION OF CARBON DIOXIDE.—Absorption of CO_2 was accomplished by means of two flasks of N/10 barium hydroxide. The first flask was given the bulk of the hydrate solution, and the second flask served as a guard against saturation of the solution over night and consequent loss of CO_2. A few drops of phenolphthalein indicated the alkalinity of the solutions. Sufficient tenth normal barium hydrate was placed in the flasks to last throughout the week. At the end of the 7-day period the excess hydrate was titrated, using oxalic acid of approximately tenth normal strength, freshly standardized, and phenopthalein indicator. The oxalic acid was standardized by titrating against the tenth normal barium hydroxide, and the latter in turn checked by titration with tenth normal HCl. At first straight barium hydroxide was used in the absorption flasks. The solubility of $BaCO_3$ in this solution was at times appreciable, however, necessitating a retitration and slight correction for certain of the flasks after standing. Consequently all later work was carried out using barium hydrate solution containing 1 gm. of barium chloride per liter. This corrected the difficulty and gave satisfactory results.

PREPARATION OF SOIL AND JARS.—The soil used was a very fine sand moderately high in well decomposed organic matter, which lent itself well to thorough mixing and subsequently to the removal of the plant roots. It was fertilized with 2000 lbs. of a 5–12–5 fertilizer per 2,000,000 lb. of soil, the fertilizer being added in the form of $NaNO_3$, KNO_3, and mono-calcium phosphate. Eight jars received 20 lb. of soil each, a ninth jar being filled with pure dry sand, through which air was aspirated similarly to the soil pots, in order to determine the CO_2 content of the air passing through the soil cultures. After carefully filling and packing the soil uniformly about the irrigator cones, the jars were joined together and the water system drawn full. They were allowed to stand for the next eight days, during which time moisture had appeared on the surfaces of all. Aspiration on the unplanted jars was then begun and continued for an 8-day period, in order to determine the behavior of the individual jars with regard to CO_2 production before being planted. The results are shown in table VII. As already indicated, pot no. 2 developed a leak in the rubber connection between the cones, and it was necessary to remove about one-fifth of the soil from the pot, in the shape of a central core, in order to repair the connection. The soil was replaced and packed as carefully as possible.

GROWTH OF PLANTS.—At the close of the 8-day run the pots showing the greatest and least production of CO_2, nos. 9 and 2 respectively, were chosen as checks and remained continuously unplanted throughout the experiment. The remaining six were planted on February 24. By March 2 all plants were well up and were thinned to ten per jar, except corn, of which five plants were allowed to grow. A layer of sand 1 inch thick was placed on the pots to reduce evaporation. Growth was very rapid for all plants during the early period. By March 28 the buckwheat was in full bloom and the soy beans beginning to bloom also. The barley showed a tendency toward prostrate growth, and the rye somewhat less with more tendency to tiller. The temperatures prevailing in the greenhouse seemed somewhat to retard the normal development and bloom of the peas. Aspiration was discontinued April 8, and the following day the plants were harvested.

METHOD OF SECURING AND HANDLING ROOTS.—After removing the tops of the plants, the roots were carefully washed out over a fine screen, using a low pressure stream of water. They were collected, freed from clinging particles of organic matter and sand by washing through several changes of distilled water until they gave solutions free of turbidity, and used for estimations of relative areas as follows. A rough approximation was made of the relative areas involved, and a corresponding quantity of a solution of methylene blue, of 50 mg. per liter strength, measured out. The roots were pressed lightly between pieces of filter paper to remove excess moisture, and dropped into the dye solution where they were kept under frequent agitation. At intervals of 10, 20, and 40 minutes, 5 cc. samples were withdrawn, diluted to a convenient range, and compared with standard color tubes made up from the original stock solution employed for the roots. From the decrease in concentration of the dissolved dye the quantity taken up was calculated, and this used as an indication of the relative areas involved, as explained later. The quantities used and the results obtained are shown in table IX. Later the roots were dried and the dry weights determined by deducting for the amount of dye absorbed.

Results

A. EFFECT OF CALCIUM SUPPLY UPON ACIDITY OF EXPRESSED SAP

The culture solutions for the nine supply bottles were made up with varying calcium content and additions of sodium carbonate. After standing for 24 hours or longer, each newly made supply was tested for reaction value. Also samples of the used solutions were caught once each week as they issued from the culture jars and their reaction values determined (for range, see table III).

The shift in reaction value of the culture solution from that contained in the supply bottle to that leaving the culture jars has usually been toward the alkaline side of the scale. A preliminary run of one week before the jars were planted also gave changes in reaction value, probably due to the activities of organisms present. The discrepancy in the P_H value of supply bottle no. 7 with the calculated value is largely due to the precipitation of calcium phosphate which occurred in the high calcium solution at P_H 6.

Table IV shows, with one exception, a slight increase in the acidity of the expressed saps with decreasing calcium supply. This is in accord with the theory earlier suggested by TRUOG (**43**,**44**), and later developed by PARKER and TRUOG (**27**), but the significance of

TABLE IV

REACTION VALUES OF EXPRESSED SAPS (GROWTH PERIOD 31 DAYS)

SUPPLY BOTTLE	CALCIUM CONTENT (PPM)	P_H VALUE OF EXPRESSED SAP		AVERAGE GREEN WEIGHT PER PLANT OF SEEDLINGS (GM.)
		Cuttings	Seedlings	
1	1000	5.77	5.60	6.1
2	100	5.55	5.57	1.5
3	10	5.35	5.50	0.4
4	1000	5.36	5.57	4.0
5	100	5.42	5.50	0.4
6	10	.....	5.42	0.3
7	1000	5.41	5.61	3.0
8	100	5.38	5.56	1.3
9	10	.....	5.51	0.3
Grown in rich soil		5.40	6.00	31.7

PLANTS GROWN IN RICH SOIL

STOCK PLANT DETERMINATIONS	P_H VALUE OF SAP
Seven months old woody stem without leaves	5.41
Somewhat younger stem without leaves	5.36
Medium stem with its leaves (duplicates)	5.02, 5.00
Tip shoots, tender stems and leaves (duplicates)	4.95, 4.95
SEEDLING PLANT DETERMINATIONS	
One month old, large, thrifty, turgid (duplicates)	5.74, 5.51
Same but wilted 24 hours (duplicates)	5.53, 5.50

the differences appears to be of doubtful value. It will be noted that the different portions of the stock plant varied as much as 0.4 of a P_H unit in the value of its juices, and individual seedlings grown side by side in soil for a period of one month showed corresponding variations of 0.2 of a P_H unit. Most of the differences accompanying the changes in calcium supply as shown in table IV are of a

lesser order of magnitude. Reference to the average green weights of the seedling plants shows that their growth was enormously affected by the differences in calcium supply, and at any given reaction of the culture solution the acidity of the sap decreases with increasing growth. Both the differences in calcium supply, however, and in the corresponding growths secured seem out of all proportion

TABLE V

REACTION VALUES OF SAPS FROM PLANTS GROWN UNDER REVERSED CONDITIONS OF CALCIUM SUPPLY

(SECOND GROWTH PERIOD 15 DAYS)

Former P_H	Direction of change	P_H value of additional growth
	Cuttings	
5.35	Low to high	5.78
5.38	Medium to high	6.21
5.77	High to low	6.02
5.36	High to low	5.70
5.41	High to low	5.73
Six weeks old cutting grown in soil		5.57
	Seedlings	
5.50	Low to high	5.99
5.42	Low to high	5.09
5.56	Medium to high	5.90
5.60	High to low	5.53
5.57	High to low	5.68
5.61	High to low	5.80
Six weeks old seedling grown in soil		5.55

to the differences in sap acidity, although the seedling grown in soil shows both the greatest growth and the least acid sap.

When the plants grown for a time under conditions of high calcium supply were changed to a low calcium solution, the resulting change in sap acidity was not toward a more acid sap, even though their growth was promptly checked by the decreased supply of calcium. Instead there seems to have been a steady march toward less acid saps, regardless of the direction of change of calcium supply. In this connection it is to be noted that the older portions of the stock plant show the less acid juices. In the light of these later

trials, it would seem that the differences first mentioned are of questionable significance, and that there is no simple direct relation between calcium supply and the acidity of the expressed sap.

B. Production of carbon dioixde by growing plant

The first carbon dioxide measurements made were discontinued after three weeks duration, because of lack of uniformity of conditions within the various pots and consequent inconsistency of results. In this first trial no auto-irrigators were used, and in spite of frequent weighings and corresponding additions of water, considerable

TABLE VI

Aspiration record of soil jars

Date	Length of period in days	Air aspirated through jars	
		Total (liters)	Daily average (liters)
February			
13–20	8	30	3.7
21–25	5	18	3.6
26–March 3	7	28	4.0
March			
4–10	7	30	4.3
11–17	7	31	4.4
18–24	7	32	4.6
25–31	7	32	4.6
April			
1–7	7	33	4.7

fluctuation in moisture was experienced, which seemed to be reflected in the quantities of CO_2 collected from the pots. Adding the water in bulk lots to the planted jars gave rise to considerable variation in distribution, while the checks went forward practically without change from day to day and week to week. A serious difficulty was encountered in the tendency of the soil to shrink away from the sides of the jar following a period of heavy transpiration. This introduced the possibility of serious alteration in the method of aspiration. It was also apparent that even with the utmost care the frequent handling of the jars necessitated by the constant weighings was not best for the plants. However, the run furnished valuable experience upon which the later technique was built. As

already explained, jars equipped with auto-irrigators were finally employed. The rates of aspiration over the eight weeks' period and the CO_2 collected from the various pots are shown in tables VI and VII *A*, *B*.

The rate of approximately 4.5 liters daily was aribitrarily chosen, after a number of trials with different rates on uniform pots of bluegrass. It seemed sufficiently rapid to give a constant drift of air downward through the jars, and also sufficiently slow to permit good uniformity of flow from the aspirator bottles.

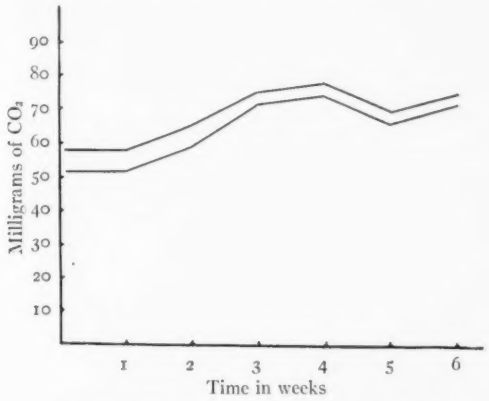

FIG. 4.—Production of CO_2 by soil check pots

In view of the divergence in CO_2 production shown during the first 8-day period between pots nos. 2 and 9, it is of interest to follow their behavior throughout the period of growth of the plants. This is shown by fig. 4. It will be seen that the initial difference is constantly lessened during the progress of the experiment, which seems to justify the method of calculation used. Thus the quantity of CO_2 given off or induced by the growing crop has been calculated by deducting the value of the air check and the average value of the two soil checks from the total production of the pot in question.

C. Root systems and root areas

In table VIII the green and dry weights of tops and roots are shown, while table IX gives the results of the attempted measure-

ments of relative root areas on the basis of the 10-minute absorption period. The figures for the longer absorption periods lead to similar results.

TABLE VII *A*

PRODUCTION OF CARBON DIOXIDE BY PLANTS GROWN IN SOIL (FIRST 8 DAYS, ALL POTS UNPLANTED)

Pot	Content	Milligrams of CO_2 collected		
		Total	From soil	Daily average
1	Dry sand	21.8	.....	.....
2	Soil	92.5	70.7	8.84
3	Soil	100.5	78.7	9.84
4	Soil	97.4	75.6	9.45
5	Soil	101.0	79.2	9.90
6	Soil	97.2	75.4	9.42
7	Soil	94.8	73.0	9.13
8	Soil	97.2	75.4	9.42
9	Soil	102.6	80.8	10.10

TABLE VII *B*

FIRST 6 WEEKS OF GROWTH PERIOD

Pot	Kind	Milligrams of carbon dioxide						
		First week	Second week	Third week	Fourth week	Fifth week	Sixth week	Total
		Total from unplanted jars						
1	Air check	21.1	24.4	22.4	23.8	24.9	26.4	143.0
2	Soil check	73.0	83.3	94.6	98.0	90.3	97.9	537.1
9	Soil check	79.0	89.6	98.0	101.6	94.2	100.8	563.2
		Increases due to plants						
3	Corn	−9.1	6.3	20.9	78.3	104.9	179.0	380.3
4	Barley	4.1	7.6	11.5	24.3	25.9	39.1	112.5
5	Buckwheat	7.4	10.1	14.1	29.8	38.1	58.3	157.8
6	Rye	3.8	9.6	13.0	23.6	28.1	54.9	133.0
7	Soy bean	15.0	21.0	29.9	48.2	62.3	75.1	251.5
8	Field pea	14.4	21.0	17.9	27.2	25.2	37.7	143.4

Table X shows what may be termed the coefficients of carbon dioxide production for the various plants secured in two ways: (1) by dividing the total CO_2 production by the figure representing the relative quantity of dry matter of the roots; and (2) by dividing

the total production of CO_2 by the quantity representing the relative root area as determined. The order of sequence of the crops is the same in either case, although the corresponding ratios differ some-

TABLE VIII

GREEN AND DRY WEIGHTS OF CULTURE PLANTS

PLANT	TOPS		DRY ROOTS (GM.)	RELATIVE DRY WEIGHTS (ROOTS)
	Green (gm.)	Dry (gm.)		
Corn	147.1	19.587	5.7082	13.30
Soy bean	69.6	16.500	1.7196	4.00
Barley	43.0	5.231	1.3893	3.24
Rye	30.8	4.323	0.5897	1.37
Buckwheat	65.8	8.857	0.4617	1.07
Field pea	31.7	4.090	0.4291	1.00

TABLE IX

ESTIMATION OF RELATIVE ROOT AREAS

Plants	Dye used (cc.)	Dye absorbed (mg.)	Relative absorption
Corn	1600	53.8	6.8
Soy bean	800	30.9	3.9
Barley	440	15.1	1.9
Rye	380	10.3	1.3
Buckwheat	200	8.3	1.05
Field pea	200	7.9	1.00

TABLE X

COEFFICIENTS OF CARBON DIOXIDE PRODUCTION

Plant	Basis of root dry matter	Basis of estimated areas
Corn	28.6	55.9
Barley	34.7	59.2
Soy bean	62.9	64.5
Rye	97.1	102.3
Field pea	143.4	143.4
Buckwheat	147.5	150.3

what among themselves. This is particularly true for corn and barley, which plants also possessed considerably larger and heavier main roots than the others.

It is not assumed by the writer that the method of dye absorption gives a true measure of the relative total areas involved. Indeed,

careful observations seem to indicate that it may more nearly approximate active absorbing areas or those areas exposed by the finer roots of the plant. The larger the diameter of the roots, the less the

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FIGS. 5, 6,—Fig. 5, soil culture jars showing method of arrangement and plants two weeks after planting: from left to right, corn, barley, buckwheat, rye, soy beans, field peas; fig. 6 same as fig. 5, four weeks after planting.

amount of dye per unit area that seemed to be concentrated on their surfaces. The smaller roots were darker following treatment with the dye. Previous trials with varying amounts of corn and soy bean roots indicated that for a given kind of root the amount of dye taken up, under the conditions of the experiment, was proportional to the surfaces exposed. There may be specific effects depending upon the nature of the roots, but at present this method of attack

TABLE XI

CARBON DIOXIDE FROM PLANTS GROWN IN QUARTZ CULTURES
(PLANTS 12 DAYS OLD WHEN ASPIRATION BEGAN)

TANK	KIND	MILLIGRAMS OF CARBON DIOXIDE				
		First week	Second week	Third week	Fourth week	Total
1.....	Unplanted	821	749	689	844	3103
		Increases due to plants				
2.....	Corn	884	927	902	1113	3826
3.....	Buckwheat	271	365	416	425	1477
4.....	Soy bean	532	630	770	829	2761
5.....	Field pea	741	789	901	617	3048
6.....	Barley	468	637	733	804	2642
7.....	Rye	354	525	765	586	2230

appears promising, and is far less laborious than that of compilations from measurements of lengths and average diameters. In connection with possible specific effects, it was noted that in both experiments the corn roots were of a lighter shade than those of the other plants after exposure to the dye.

D. PLANTS GROWN IN QUARTZ CULTURES

Tables XI–XIV give the results secured from the plants grown in quartz cultures. In this case there was not the tendency toward prostrate growth previously described, but both rye and barley tillered freely. The growth of the rye and buckwheat seemed less vigorous than that of the other plants. There was always free culture solution standing in the bottoms of the metal containers.

The results obtained with the quartz cultures are in general agreement with those secured from the soil cultures, although less

consistent than the latter. A quite unexpected result was the very much greater production of CO_2 by the quartz cultures. A study of the tables shows that both the plants and the culture medium

TABLE XII

GREEN AND DRY WEIGHTS OF QUARTZ CULTURE PLANTS

PLANT	TOPS		DRY ROOTS (GM.)	RELATIVE DRY WEIGHTS (ROOTS)
	Green (gm.)	Dry (gm.)		
Corn	140.2	14.872	3.249	12.94
Buckwheat	16.1	2.198	0.251	1.00
Soy bean	43.0	8.503	2.171	8.65
Field pea	24.2	2.988	0.892	3.55
Barley	43.7	4.860	1.392	5.55
Rye	23.7	3.590	1.025	4.08

TABLE XIII

ESTIMATION OF RELATIVE ROOT AREAS

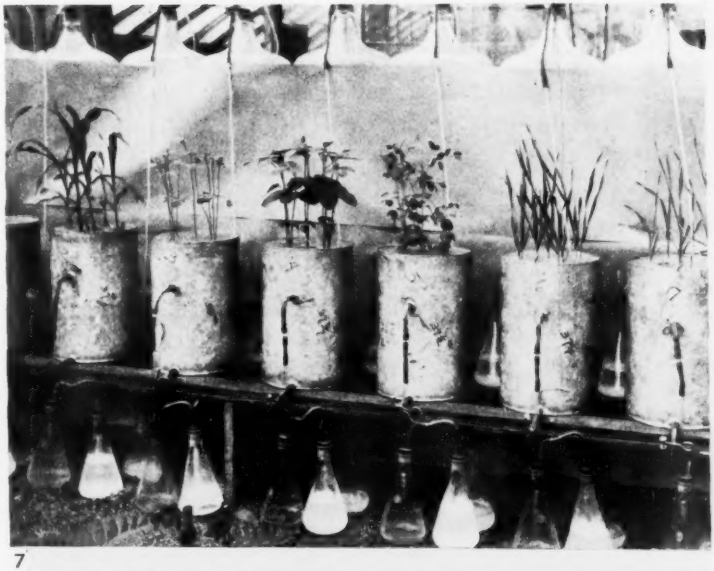
Plants	Dye used (cc.)	Dye absorbed (mg.)	Relative absorption
Corn	1600	33.3	5.8
Soy bean	1000	23.2	4.0
Barley	720	14.7	2.5
Rye	640	13.2	2.3
Field pea	400	8.8	1.5
Buckwheat	200	5.8	1.0

TABLE XIV

COEFFICIENTS OF CARBON DIOXIDE PRODUCTION

Plant	Basis of root dry matter	Basis of estimated areas
Corn	295	660
Soy bean	319	690
Barley	476	1060
Rye	546	970
Field pea	858	2030
Buckwheat	1477	1477

(solution) contributed to the increase. The reason for this behavior is not entirely clear. The number of plants per culture was the same in either case, and the difference in size of containers was slight. It is not possible to account for the differences on the basis of loss by diffusion from the soil jars.



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FIGS. 7, 8.—Fig. 7, air-tight containers with plants sixteen days after planting: from left to right, corn, buckwheat, soy beans, field peas, barley, rye; fig. 8 same as fig. 7, four weeks after planting.

E. Permeability and specific absorptive nature of plant roots

The rôles played by permeability and the specific adsorptive nature of root colloids in the absorption of minerals by plants are still unsolved problems. The field is a very complicated one, and difficult to submit satisfactorily to experimental test. In trials with the absorption of copper salts from solution by different kinds of roots, it was noted that barley seemed always to take up more of this element in a given length of time than equal masses of certain other kinds of roots. Following this lead, an attempt was made to measure the relative quantities of calcium and potassium absorbed during very brief intervals of five to ten minutes from equivalent concentrations of their bicarbonates. Most of the plants tried seemed to remove both elements in equivalent amounts, but barley and bluegrass took up approximately three times as much calcium as potassium, whereas rye took up twice as much potassium as calcium. The extent to which ion exchange may have entered in was not determined, and the experiments were not repeated. It is very difficult to know when, if ever, plant roots are free from adsorbed materials on their surfaces, and the significance of the results is therefore obscure. Nevertheless the possibility of importance of these factors cannot be denied, and it is hoped that future work will throw more light on their significance.

Discussion

An effort has been made to consider various factors that may be involved in the absorption of mineral elements by plants, and evidence bearing upon certain of these factors is presented. In connection with the relation of calcium supply to the acidity of the plant sap, if one of the chief functions of calcium is to neutralize by-product acids in the plant, it seems reasonable to suppose that limiting the plant's supply of this element would result in appreciable increases in the acidity of the expressed sap. Just how great such increases would be is somewhat problematical. Probably they would not be pronounced, yet it would seem that they should be capable of measurement and of greater degree than the moderate variations commonly observed in normal plants. It is apparent that the

results herein reported do not bear out, although they do not necessarily disprove, the theory advanced by TRUOG and his co-workers. It is true that analyses were not made to show that the plants actually took up more calcium from the high calcium solutions, but this was done by NEWTON with results in general agreement with those given here. In this work the tomato was used as a culture plant because of the large quantity of juices normally present in its tissues, and because of the possibility of securing a large number of cuttings from a single plant, thus minimizing the differences so frequently encountered due to individual variation.

With respect to the relation of calcium to organic acids in plants, it is interesting to examine some of the ideas prevalent in the literature. As already stated, PARKER and TRUOG suppose one of the chief functions of calcium to be the neutralization and removal of excessive and harmful acids formed as by-products during normal growth processes. On the other hand, THATCHER (**39**) believes the deposition of calcium oxalate crystals to be a device for the avoidance of excessive calcium in the plant juices. This latter view is held also by GERHARDT (**9**) and by STAHL (**35**), these investigators associating calcium oxalate formation with guttation, and the presence of mycorrhiza as a means of removal and prevention of excessive salts, especially those of calcium. Whatever their true rôles may be, it appears that present knowledge is insufficient to support a statement of direct functional relation between calcium and organic acids in plant juices.

The results dealing with carbon dioxide measurements are in general accord with those reported by other writers. LAU (**18**) found a greater production of CO_2 in soils growing potatoes and lupines than in those growing cereal crops, while STOKLASA and ERNEST (**37**) found buckwheat, oats, and rye to produce more CO_2 per unit of dry weight of roots than wheat or barley. TRUOG (**45**) apparently considers the differences in CO_2 production by plant roots as insufficient to account for the differences in growth response commonly observed, although the solvent action of CO_2 at root surfaces is by no means ignored. He emphasises (**42**) the fact that the conditions obtaining in root cell and root hair surfaces have never been duplicated in laboratory experiments with CO_2. Produc-

tion of CO_2 is apparently an expression of metabolic activity, and different species of plants differ considerably in this respect. This is also true when relative root masses and recoverable root areas are considered. Buckwheat shows a very meager root system in proportion to its total dry weight, yet it gives off relatively large quantities of CO_2, which must result in uniformly higher concentrations of carbonic acid about its absorbing roots than is the condition with many other plants. The work of SCHLOESING (**32**) suggests the possibility of an increasing rate of solubility of certain minerals with increasing concentrations of CO_2. Continued removal of the dissolved substances by the plant would result in a still greater effect of the CO_2 excreted. It is at least interesting to observe that the order of sequence of the crops with respect to the intensity of CO_2 production, as shown in table X, is very similar to that shown by actual farm experience in their ability to grow on infertile soils. It would doubtless be erroneous to attribute differences in the native capacity of plants to absorb mineral elements from the soil to differences in carbon dioxide production alone. More probably there are many factors involved of which carbon dioxide is an important one.

It is unfortunate that we have no accurate knowledge of the production and distribution of root hairs for the various kinds of plants growing under normal conditions in soils. Consequently we are forced to base opinions upon the recoverable portions of the root systems only, which may not necessarily be proportional to total absorbing surfaces. In his report on corn and the sorghums, MILLER states that, judging from the number of secondary roots found, certain sorghums have a root system twice as efficent as corn in the absorption of water from the soil. Comparative extent of root systems is probably important in determining differences in absorption by plants otherwise similar in metabolism, composition, and growth, but that it cannot be the only factor bearing upon this point, or even a dominant one under all conditions, is readily seen by a comparison of the root systems of two such plants as barley and buckwheat. The production and operation of root hairs, together with permeability and colloidal absorptive effects, are in need of much further investigation.

Summary

Studies were made of the effect of varying calcium supply upon the acidity of the expressed sap of tomato plants, and of the production of carbon dioxide by the root systems of common crop plants. The outcome of these have been as follows:

1. Limiting the plant's supply of calcium, even to the point where cessation of growth and death resulted, did not increase the H-ion concentration of the expressed sap in significant degree.

2. When plants supplied with a high calcium solution were transferred to one of low calcium content the expressed saps did not show a corresponding increase in acidity, although the nature of the change was sufficiently radical to retard their growth.

3. Irrespective of the direction of change of calcium supply, the saps of the plants so treated became somewhat less acid.

4. Moderate variations in reaction values were found to occur in the expressed saps from different individuals and from different portions of the same plant.

5. The stock plant from which the cuttings were taken showed a decreasing acidity gradient with increasing age of the tissues.

6. Considerable differences were found in the production of carbon dioxide by the roots of different species of plants.

7. Consideration of the relative size and area of root systems in connection with CO_2 output leads to the conclusion that carbon dioxide is an important factor in aiding certain kinds of plants to secure essential elements from the soil.

8. The production of carbon dioxide, whether from the standpoint of unit of dry matter of roots or from that of unit area of root system, as estimated in these experiments, has been greater for buckwheat and rye than for barley and corn.

9. The results are in general agreement with practical experience as to the ability of plants to grow satisfactorily on infertile soils.

The writer desires to acknowledge his indebtedness to Dr. C. A. SHULL and Dr. S. V. EATON of the Department of Botany for their kindly interest and many helpful suggestions throughout the course of the experiments.

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THE HEMISPHAERIACEAE OF BRITISH GUIANA AND TRINIDAD

F. L. Stevens and H. W. Manter

(with plates xviii–xxi)

The Hemisphaeriaceae, or "fly-speck" fungi, are chiefly tropical and are strictly superficial upon the surface of leaves. They either lack a true mycelium or it is so inconspicuous as to escape observation. The appearance of the flattened, circular, usually blackened thyriothecia (perithecia) on the leaf has led to the popular term "fly-specks." The studies in the present paper are based on material collected by the senior author in British Guiana and Trinidad during the summer of 1922. General observations in regard to the collecting, together with a map of the regions covered, as well as a list of the field numbers with places and dates of collection may be found in a previous paper (10). The original slides, drawings, and specimens upon which these studies are based are deposited in the herbarium of the University of Illinois. The photographs in the present paper are by A.G. Eldridge.

The general typical appearance of the thyriothecia spotting the leaf surface is shown in figs. 1 and 3. The central region of old thyriothecia sometimes falls away, giving the appearance shown in fig. 2. These disclike bodies are easily removed from the leaf, and are readily studied in temporary water mounts, where the fruit body can be cut or crushed to reveal its asci and spores. Light staining with iodine may be advantageous. Permanent mounts of the thyriothecia are easily made by the celloidin method (9). The thyriothecia of the most common group of these fungi are carbonaceous and opaque, except for a narrow peripheral region which is blue-green; others may be brown in color. Old thyriothecia often appear entirely carbonaceous. The extremely variable and beautiful conditions of the spores and asci lend especial interest to the group. The taxonomy of the group is somewhat confused, and some history of the changes it has undergone is important.

The Hemisphaeriaceae are very similar to the Microthyriaceae. The latter is a very large group of some 32 genera (of which *Asterina* alone contains over 100 species), also chiefly tropical in distribution, and likewise possessing disclike, superficial thyriothecia. In fact, the two groups were not distinguished for a long time, both being united under the Microthyriaceae.

Apparently the first attempt to separate the blue-green "fly-specks" from the Microthyriaceae was made by VON HÖHNEL in 1910 (**22**). In commenting upon *Micropeltis applanata*, he pointed out that this species differed from the ordinary species of the Microthyriaceae in that the thyriothecia were not formed as inverse fruit bodies. He accordingly listed *Micropeltis* and *Scolecopeltis* as disc-shaped Sphaeriaceae. In 1912 (**23**), he discarded from *Micropeltis* many of the former species, on the basis of his former distinction between them and the Microthyriaceae. He stated that many of the so-called *Micropeltis* species, especially the smaller forms with radial structure, belong to the Microthyriaceae proper. Before that time 69 species of *Micropeltis* had been described. Of these, VON HÖHNEL retained only 23 as true *Micropeltis* species, and assigned the others to various genera among the Microthyriaceae and lichens.

In 1913 THEISSEN (**16**) established a distinction between the Microthyriaceae and the group represented by *Micropeltis* by erecting a new family, Hemisphaeriaceae, for the latter type; the Microthyriaceae being characterized by inverse and radiate thyriothecia, the Hemisphaeriaceae by non-inverse and non-radiate thyriothecia. Furthermore, THEISSEN united all the species with disc-shaped thyriothecia into a new order, which he named Hemisphaeriales (**16**). In this order, in addition to the previously mentioned families, he included the Trichopeltaceae, which possess disc-shaped thyriothecia but also a vegetative thallus. A key to these families superficial on the host would then be (according to THEISSEN and SYDOW):

Thyriothecial membrane radial
Thallus thready or lacking ..... Microthyriaceae
Thallus membranous, radial ..... Trichopeltaceae
Thyriothecial membrane not radial ..... Hemisphaeriaceae

THEISSEN then proceeded to divide the Hemisphaeriaceae into subfamilies based on the structure of the thyriothecial membrane.

The Dictyopeltineae (Dictyopelteae) possess a netlike covering blue-green or blue-black in color, and the Thrausmatopeltineae (Thrausmatopelteae) a pseudo-parenchymatic covering, easily breaking into pieces and yellow to brown-black in color. A third subfamily later named by THEISSEN (**17**) is Plochmopeltineae, with thyriothecial membrane of meandering, plectenchymatic structure, and with a netlike mycelium without hyphopodia. STEVENS and GUBA have added a fourth subfamily (**11**), with "thallus that of the Thrausmatopeltineae, asci solitary, naked, without a covering membrane."

Key to subfamilies of Hemisphaeriaceae (modified after THEISSEN and SYDOW):

Thyriothecial membrane open netted, blue-green, without true mycelium ..... Dictyopeltineae Theiss.

Thyriothecial membrane pseudo-parenchymatic, brown, without true mycelium

Asci covered ..... Thrausmatopeltineae Theiss.

Asci naked ..... Gymnopeltineae Stevens and Guba

Thyriothecial membrane meandering-plectenchymatic, mycelium netlike, no hyphopodia ..... Plochmopeltineae Theiss.

The Dictyopeltineae are by far the most common, with *Micropeltis* as perhaps the most common genus. As has been shown, this genus in the past has been overworked as a pigeon hole for many fungi of the general characteristics of the group. Now, however, it has been conveniently divided into a number of genera. THEISSEN's step in placing all radiate thyriothecia in the Microthyriaceae was perhaps the most valuable delimination.

As early as 1889, SPEGAZZINI (**5**) established the genus *Scolecopeltis*, which is distinguished from *Micropeltis* chiefly in possessing filiform, many-septate spores which tend to fall apart into their component cells. This distinction is not entirely satisfactory, as it requires an arbitrary decision as to what constitutes a filiform spore. The many-septate condition of the spore is highly variable in spores of more than a few cells. The loose connection between the cells in the spore, considered together with the filiform character, however, makes *Scolecopeltis* quite definitely distinct from *Micropeltis*. The type species of *Scolecopeltis* is aparaphysate. It has seemed best in this paper to erect for all species which otherwise agree with

Scolecopeltis, but which possess paraphyses, a new genus, *Scolecopeltidium*.

The presence or absence of paraphyses seems to be a constant character, and a valid distinction upon which to separate genera. In 1913 SYDOW (**12**) utilized this character to distinguish a new genus, *Micropeltella*, without paraphyses, from *Micropeltis*, the type of which possesses paraphyses. *Micropeltella* at once adopted many of the old species of *Micropeltis*.

When the number of cells in the spores of these fungi is small, it usually is a constant character; hence, spore septation has been used to separate a few genera from *Micropeltis*. Those with 1-celled spores were allotted to *Dictyothrina* by THEISSEN (**16**); 2-celled spores to *Dictyothyrium* by THEISSEN (**14**); and 3-celled spores to *Dictyothyriella* by REHM (**17**). This last genus includes some of the most common members of the group, and numerous previously described *Micropeltis* species. *Dictyopeltis* Theiss. (**16**), with 2-celled spores, is distinct from *Dictyothyrium* in that it has no ostiole, but when ripe the whole thyriothecium breaks away from its rim.

Recently, SPEGAZZINI has separated several genera on the basis of the possession of stellate ostioles instead of the normal circular ostioles. The three genera created on such a basis are:

STELLATE OSTIOLE		ROUND OSTIOLE	
Micropeltidium	separated from	*Micropeltis*	(**6**)
Parapeltella	separated from	*Micropeltella*	(**6**)
Scolecopeltella	separated from	*Scolecopeltis*	(**7**)

It seems somewhat doubtful whether this stellate or round condition of the ostiole is a sufficiently definite character to be of taxonomic value. In all the material observed in the present studies, none of the thyriothecia appeared to possess what could be called a characteristically stellate ostiole. It is true, however, that ostioles become quite irregularly circular, due to a tendency of their borders to fray or slightly split (figs. 55–57). If the very definite stellate condition illustrated by SPEGAZZINI (**6**) is a constant and permanent form, then it may well be considered as a valid and useful distinction. Since no specimens of such a type were seen, these three genera of SPEGAZZINI are accepted and included in the key to the genera of the

Dictyopeltineae. Curiously enough, SPEGAZZINI, in his key to the Dictyopeltineae (**7**), lists his genus *Parapeltella* as with a round ostiole, and separates it from *Micropeltella* by its clavate spores, a new distinction which hardly seems justified. Since the type description of the genus holds validity, this later discrepancy is doubtless due to an error in the publication of his key.

There appears to be another point of confusion in the consideration of SPEGAZZINI'S genus *Micropeltidium*. As noted, this genus was separated from *Micropeltis* in 1919 only on the basis of its stellate ostiole. In the original description the genus was characterized as with paraphyses, and the type species (*Micropeltis tonduzii*) is densely paraphysate. It seems clear, then, that the presence of paraphyses is a character of the genus, as, indeed, otherwise it would correspond with *Parapeltella*. In 1923, however, SPEGAZZINI (**7**) seems to describe *Micropeltidium* again as a new genus, and here he points out that it is aparaphysate. The two species listed under it in 1923 are aparaphysate. Accepting the earlier and type descriptions of the genera involved, these two species seem to belong to *Parapeltella*.

This confusion in the conception of *Micropeltidium* has involved also another genus, *Metapeltella*, which SPEGAZZINI named in 1923 (**7**) in connection with his genus *Micropeltidium*, named at that time. *Metapeltella* is like *Micropeltidium*, except that the spores are clavate with the tip cell much larger. Since the genus is based on SPEGAZZINI'S second *Micropeltidium*, *Metapeltella* is aparaphysate with stellate ostiole. SPEGAZZINI himself remarks that the spores of *Metapeltella* are like those of *Parapeltella*. As has been shown, the second *Micropeltidium* could not be distinguished from SPEGAZZINI'S earlier *Parapeltella*, and this agreement of spore shape makes the identity of the two genera (*Metapeltella* and *Parapeltella*) even more perfect. From a careful study of the descriptions of these genera, it does not seem possible to separate *Metapeltella* from *Parapeltella* (as originally described), while the second *Micropeltidium* is separated from *Parapeltella* only on the basis of its possession of fusoid instead of clavate spores, and from *Micropeltella* only by its stellate ostiole. Whether genera should be separated on such considerations seems doubtful, but as no material of these species or groups was encountered in the

material examined by us, the genera are included in the following key. SPEGAZZINI's second *Micropeltidium* is designated as *Micropeltidium* no. 2.

One other puzzling point remains in regard to SPEGAZZINI's genus *Micraspidium*. No reference could be found to this genus except in SPEGAZZINI's key to the Dictyopeltineae in 1923. According to this key, *Micraspidium* cannot be distinguished from the earlier *Micropeltidium*, which, of course, is not represented in the key except as the second *Micropeltidium* (of different characters). Without knowledge of the type species of *Micraspidium* no definite statement can be made, but it may be significant that if the earlier *Micropeltidium* had been published as *Micraspidium*, most of the seeming contradictions in SPEGAZZINI's 1923 paper would be cleared up.

A genus, *Micropeltopsis*, described by EARLE[1], introduces a new distinguishing character. *Micropeltopsis* is separated from *Micropeltis* by possessing a white membranous border or halo around the thyriothecia. There are two objections to such a separation. First, the present studies seem to show that the halo, sometimes prominent, may be reduced in old thyriothecia of the same species, and in many cases even when present it can easily be lost in removing the thyriothecia from the leaf. Second, it is a character rarely mentioned in old descriptions, and the whole group would be of uncertain position until re-examined. *Micropeltopsis*, therefore, is not recognized in the following key.

All of these genera of the Dictyopeltineae are characterized by a netlike thyriothecial covering, and also by a blue-green color. THEISSEN in 1913 (**15**) noted this correlation, although the important character is the type of structure. The blue-green color is given by THEISSEN as accompanying all of the Dictyopeltineae known. One form was found in the present studies, however, which clearly possessed a coarse, open netted thyriothecial covering, but was a rich brown. This form is included under the Dictyopeltineae as a new genus, *Theciopeltis*.

[1] SPEGAZZINI (**7**) credits EARLE with describing this genus with the characters noted, but no trace of the original description could be found. The genus is also unknown at the New York Botanical Gardens, where most of the EARLE's specimens are deposited.

Following generic characters as outlined, a key to the genera of the Dictyopeltineae (based on the key by THEISSEN and SYDOW **19**) is given as follows:

Thyriothecial membrane open netted
Thyriothecial membrane blue-green
Spores 1-celled ..... *Dictyothyrina* Theiss.
Spores 2-celled
Ostiole present ..... *Dictyothyrium* Theiss.
Ostiole absent ..... *Dictyopeltis* Theiss.
Spores 3-celled ..... *Dictyothyriella* Rehm
Spores 4 to many-celled
Paraphyses present
Ostiole round ..... *Micropeltis* Mont.
Ostiole stellate ..... *Micropeltidium* Speg.
Paraphyses absent
Ostiole round ..... *Micropeltella* Syd.
Ostiole stellate
Spores clavate ..... *Parapeltella* Speg.
Spores fusoid ..... *Micropeltidium* no. 2 Speg.
Spores filiform
Paraphyses present ..... *Scolecopeltidium* Stevens and Manter
Paraphyses absent
Ostiole round ..... *Scolecopeltis* Speg.
Ostiole stellate ..... *Scolecopeltella* Speg.
Thyriothecial covering brown ..... *Theciopeltis* Stevens and Manter

Each genus represented in the material studied will be considered later, together with keys to the species.

DICTYOTHYRIUM Theiss. Oster. Bot. Zeitschr. **62**:277. 1912

This genus has only a few species. It is clearly separated from *Micropeltis* which it resembles except that it has 2-celled spores. *M. corruscans* Rehm (**3**) is described as having 1-septate spores, so that we consider this species also as *Dictyothyrium*.

Spores clavate ..... *D. subcyaneum* (Ellis and Mart.) Theiss.
Spores cylindrical
Paraphyses present
Asci 2-spored ..... *D. disporum* Stevens and Manter
Asci with variable number of spores
Epiphyllous
Asci 90 μ in length ..... *D. chalybaeum* (Rehm) Theiss.
Asci 125–170 μ ..... *D. leucopterum* Syd.
Mostly hypophyllous ..... *D. giganteum* Syd.
Paraphyses absent
Asci 8-spored ..... *D. corruscans* (Rehm), n. comb.

1. **Dictyothyrium disporum** Stevens and Manter, n. sp. (fig. 8) —Thyriothecia amphigenous, mostly hypophyllous, 535 μ in diameter, margin blue-green; ostiole round, up to 40 μ in diameter; asci obclavate, stipitate, 138×33 μ, always 2-spored; paraphyses filamentous, crooked, copious; spores large, cylindrical, sometimes somewhat pointed at ends, especially when young, obtusely rounded at ends when mature, 1-septate, constricted, cells usually equal, basal cell sometimes slightly longer, spores 70–95×10–12 μ.

On unknown member of the Menispermaceae, British Guiana: Rockstone, July 16, *435*.

This fungus is nearest *D. leucopterum* Syd., but differs from it in being mostly hypophyllous and in always having 2-spored asci. It differs from *D. giganteum* Syd. by having larger and 2-spored asci.

2. DICTYOTHYRIUM GIGANTEUM Syd. Phil. Jour. Sci. **9**:C. 178. 1914

On *Serjania paucidentata*, British Guiana: Kartabo, July 23, *586*.

This fungus agrees with the preceding species in all measurements and in spore septation. It disagrees, however, in a number of characters. Thus, asci were found with 1–3–4–6–8 spores. Great variation was found in the size and appearance of spores in the same ascus. Owing to the small amount of material available, the fungus has been accorded to the species *D. giganteum*, which it most closely resembles. A study of further material, if possible, would perhaps establish it as a new species.

3. DICTYOTHYRIUM LEUCOPTERUM Syd. Ann. Myc. **14**:93. 1916

On *Eperua* sp., British Guiana: Wismar, July 14, *271*.

DICTYOTHYRIELLA Rehm, Broteria **12**:92. 1914

Dictyothyriella is perhaps of more frequent occurrence than any other genus in the group. Its 2-septate spores are quite constant, and separate it from *Micropeltis*. BEELI (**1**) does not seem to recognize *Dictyothyriella*, as he describes two *Micropeltis* species (*M. wildemanii* and *M. dubia*) with 2-septate spores. We are including these species in the key under *Dictyothyriella*. The genus is also apparently accidently omitted from SPEGAZZINI'S key to the *Dictyopeltineae* in 1923.

Members of the same species of *Dictyothyriella* seem to grow on a great variety of hosts. Not only is the same species found on widely different hosts, but the same species appears to occur in widely differ-

ent parts of the world. This condition of host relationship and distribution seems to be characteristic of the Hemisphaeriaceae in general. While there may be biological specialization among the "fly-specks," as the fact that they are not found indiscriminately on all leaves in their neighborhood seems to indicate, it is not in many cases manifested morphologically.

Extreme morphological variation is often found within a single species of *Dictyothyriella*. As a result of this variation it is often very difficult to separate species, and some species necessarily embrace a large range of characters, and thus many examples of these species are found. For example, *D. heterosperma* has been found to exhibit nearly all possible variations, even within a single thyriothecium.

Species have been separated as distinctly as possible in the key, but it should be remembered that this key, as well as other keys in this paper, is sometimes forced to resort to characters of rather weak significance. The keys are entirely artificial. It is hoped that they will prove a convenient guide in determining species, but they cannot be considered as ultimate, nor even as bringing out the most important differences in every case. In a few cases no distinction could be discerned between two species from the descriptions, which are frequently inadequate. In such cases the two species are listed together.

Paraphyses lacking
  Asci 8-spored
    Spores not constricted.....*D. microsperma* (Syd.), n. comb.
    Spores only slightly constricted.....*D. wildemanii* (Beeli), n. comb.
    Spores strongly constricted
      Asci 60–75 μ long.....*D. harmsiana* (P. Henn.), n. comb.
      Asci 85–120 μ long.....*D. camarinensis* (Syd.), n. comb.
  Asci 2–4–8-spored
    Spores rarely 3-septate, 65–75 μ.....*D. megasperma* (Syd.), n. comb.
    Spores rarely 1-septate, 36–48 μ.....*D. dubia* (Beeli), n. comb.
Paraphyses present
  Asci 8-spored
    Spores not over 20 μ
      Hypophyllous.....*D. biseptata* (v. Höhn.) Theiss.
      Epiphyllous
        Spores obclavate.....*D. microspora* (Speg.) Theiss.
        Spores clavate or oblong
          Asci 30–35 μ.....*D. bauhiniae* (Rehm), n. comb.
          Asci 45–55 μ.....*D. trewiae* Syd.
D. semecarpi (Syd.), n. comb

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Species have been separated as distinctly as possible in the key, but it should be remembered that this key, as well as other keys in this paper, is sometimes forced to resort to characters of rather weak significance. The keys are entirely artificial. It is hoped that they will prove a convenient guide in determining species, but they cannot be considered as ultimate, nor even as bringing out the most important differences in every case. In a few cases no distinction could be discerned between two species from the descriptions, which are frequently inadequate. In such cases the two species are listed together.

Paraphyses lacking
- Asci 8-spored
  - Spores not constricted ..... *D. microsperma* (Syd.), n. comb.
  - Spores only slightly constricted ..... *D. wildemanii* (Beeli), n. comb.
  - Spores strongly constricted
    - Asci 60–75 μ long ..... *D. harmsiana* (P. Henn.), n. comb.
    - Asci 85–120 μ long ..... *D. camarinensis* (Syd.), n. comb.
- Asci 2–4–8-spored
  - Spores rarely 3-septate, 65–75 μ ..... *D. megasperma* (Syd.), n. comb.
  - Spores rarely 1-septate, 36–48 μ ..... *D. dubia* (Beeli), n. comb.

Paraphyses present
- Asci 8-spored
  - Spores not over 20 μ
    - Hypophyllous ..... *D. biseptata* (v. Höhn.) Theiss.
    - Epiphyllous
      - Spores obclavate ..... *D. microspora* (Speg.) Theiss.
      - Spores clavate or oblong
        - Asci 30–35 μ ..... *D. bauhiniae* (Rehm), n. comb.
        - Asci 45–55 μ ..... *D. trewiae* Syd.

D. semecarpi (Syd.), n. comb

Spores 35 μ in length..... *D. trimera* (Sacc.), n. comb.
Spores 38–62 μ in length..... *D. macromera* (Syd.), n. comb.
Asci 4-spored
Tip cell of spore pointed..... *D. coerulescens* (Rehm), n. comb.
Asci with variable number of spores
Tip cell of spore pointed
Asci usually 4 or 6-spored, rarely 2-spored
Spores 30–60 μ..... *D. guianensis* Stevens and Manter
Asci usually 2-spored, sometimes 4. *D. philodendri* Stevens and Manter
Asci 4–8-spored, usually 8, rarely 2
Spores 50–72 μ..... *D. heterosperma* Syd.
Tip cell of spores not pointed
Asci 2–4–6-spored
Spores 60–90–100 μ long..... *D. vismiae* Stevens and Manter
Asci 2–4–6–8-spored
Spores 30–38 μ long..... *D. mucosa* Syd.

4. **Dictyothyriella guianensis** Stevens and Manter, n. sp. (figs. 10–13).—Thyriothecia epiphyllous, either gregarious or somewhat scattered, 590–850 μ in diameter, margin blue-green, netted; ostiole round, 25–45 μ in diameter; asci cylindrical, short stipitate, 4–6–8-spored, rarely 2-spored, usually 4–6-spored, 100–155×15–25 μ; paraphyses copious, filamentous, crooked; spores typically 2-septate, constricted, cells easily separating, tip cell always larger and pointed, 30–60×5–8 μ.

On *Costus* sp., British Guiana: Rockstone, July 16, *1005;* on *Pesequeria latifolia*, British Guiana: Kartabo, July 22, *534;* on *Maurita* sp., Trinidad: Cumuto, August 16, *1007;* on *Philodendron* sp., British Guiana: Kartabo, July 23, *1006;* on *Licenia* sp., *Tabernaemontana* sp., and *Bauhinia* sp., British Guiana: Rockstone, July 17, *479, 473, 469;* on unknown member of the Marantaceae, British Guiana: Kartabo, July 21, *505;* on unknown member of the Apocynaceae, British Guiana: Tumatumari, July 11, *163;* on unknown host, British Guiana: Kartabo, July 17–23, *530, 606, 608;* Tumatumari, July 8–11, *993, 155;* Wismar, July 14, *275*.

This fungus showed considerable variation, and often tended to approach *D. heterosperma*, which it closely resembles, especially in spore shape. *D. guianensis*, however, is always epiphyllous (although this cannot be considered a very significant character), usually has less than eight spores in the ascus, and all measurements are usually less than those of *D. heterosperma*.

5. **Dictyothyriella philodendri** Stevens and Manter, n. sp. (figs. 24–26).—Thyriothecia epiphyllous, 960 μ in diameter, ostiole round, 25 μ in diameter; asci clavate to elongate, often slightly curved, stipi-

tate, 150–155×17–18 μ, 2–4-spored; paraphyses filamentous, crooked, not longer than asci; spores 2–4-septate, deeply constricted, cells easily separating, sometimes separate in the ascus, terminal cell elongated-pyriform with narrow distal region, longer than other cells, spores 50–60 μ×7–9 μ.

On *Philodendron* sp., British Guiana: Kartabo, July 23, *1006*, *479*.

This fungus is close to *D. guianensis*. It differs in possessing usually 2-spored asci, asci and spores more elongated, and spores frequently 3 or 4-septate.

6. DICTYOTHYRIELLA HETEROSPERMA Syd., Ann. Myc. **15**:231. 1917 (figs. 14–23)

On *Coccoloba* sp., British Guiana: Kartabo, July 23, *597;* on unknown member of the Myrtaceae, British Guiana: Rockstone, July 17, *486;* on unknown member of the Anonaceae, with *Micropeltella minima*, British Guiana: July 24, *1011;* on unknown host, British Guiana: Kartabo, July 22–24, *571*, *600*, *1003*, *666*.

In his original description, SYDOW pointed out the high degree of variability of this fungus. Some of these specimens showed an even greater degree of variability. Thyriothecia were occasionally amphigenous, but commonly hypophyllous. The asci contained almost any number of spores up to eight. It can be seen that numerous characters must be considered to separate this species from *D. guianensis*.

An unusual condition was observed in one of the specimens, where the ascus frequently had four or six spores. One ascus was found with three large spores and one spore near the tip considerably dwarfed (fig. 16). The very marked difference between the size of this single spore and the others occupying the ascus seems to give a clue to the variability of the number of spores in the asci of this group, since it appears that some spores fail to develop, and finally degenerate, owing perhaps to competition among the spores in a single ascus.

Specimen no. 600 (figs. 18–22) revealed a remarkable variation, not only in spore septation, but also in the number of spores in the ascus. Thus in a single thyriothecium, one ascus contained four spores all many-septate (4–6-celled), another ascus had only three spores one of which was 4-celled, and another ascus had eight spores of normal septation. One ascus was found with only one spore, a number of asci with two spores, while a 4 or 6-spored condition was most common. The measurements of all parts coincided perfectly with those of *D. heterosperma*. Fortunately, specimens of this species identified by SYDOW were available for comparison. The principal difference noted was in the number of spores in the asci. In the authentic *D. heterosperma*, either six or eight spores were almost invariably present. This difference, however, does not seem to be sufficient to exclude the present specimens, and they are accordingly identified as *D. heterosperma*. Associated with this fungus (no. 600) was *Micropeltella minima*.

7. **Dictyothyriella vismiae** Stevens and Manter, n. sp. (figs. 27–29).—Thyriothecia epiphyllous, 600–700 μ in diameter, margin blue-green; ostiole round, 40 μ in diameter; asci obclavate to cylindrical, sessile or very short stipitate, 4–6-spored, paraphyses copious, filamentous, crooked; asci 140–160×20–35 μ; spores 2-septate, deeply constricted, cells nearly equal and easily separating, tip cell not pointed, cells nearly oval in shape, spores 60–90×12–16 μ.

On *Vismia* sp., British Guiana: Rockstone R. Ry., July 15, *1014;* on *Coccoloba* sp., British Guiana: Rockstone, July 17, *476;* on unknown member of the Loranthaceae, British Guiana: Kartabo, July 21, *540;* on unknown host, British Guiana: Kartabo, July 23, *591.*

This fungus differs from *D. heterosperma* and *D. guianensis* in having all the cells of the spore nearly equal, and with the tip cell bluntly rounded instead of pointed. Material from nos. *540*, *476*, and *591* sometimes showed spores so large that these numbers were at first allotted to a new species. As in all other respects they were similar to *D. vismiae*, and as even spore measurements frequently agreed, they are included in that species even though some of the spores were 50–150 μ in length and asci sometimes 250 μ long.

MICROPELTIS Mont., Pl. Cell. de L'ile de Cuba, p. 325. 1842

The following species of *Micropeltis* are described having thyriothecia with radial coverings, and are therefore considered as excluded from the Hemisphaeriaceae: *M. sirie* Rac., *M. bambusae* Pat., *M. heteropteridis* Theiss. The two following species are inadequately described: *M. stigma* Cooke, *M. asterophora* B. and Br. Species described as without paraphyses are considered as belonging to *Micropeltella.*

Thyriothecia blue-green
- Spores with constant number of septa
  - Spores with 3 septa
    - Asci 4-spored ..... *M. tetraspora* Stevens and Manter
    - Asci usually 8-spored
      - No true ostiole ..... *M. pitya* Sacc.
      - Ostiole present
        - Asci 6–8-spored ..... *M. marginata* Mont.
        - Asci 8-spored
          - Hypophyllous ..... *M. albo-ostiolata* P. Henn.
          - Epiphyllous
            - Asci clavate ..... *M. depressa* Cke. / *M. oleandri* Br. and Har.
            - Asci oblong ..... *M. pometiae* Rehm
          - Amphigenous ..... *M. vagabunda* Speg.

Spores with 4 septa ..... *M. clavigera* Sacc.

Spores with 5 septa

Spores constricted ..... *M. applanata* Mont.

Spores not or little constricted

Asci 8-spored ..... *M. similis* Syd.

Asci 4–6–8-spored ..... *M. acalyphae* Syd.

Spores with 8 septa ..... *M. viridiatia* Wint.

Spores with variable number of septa

Spores with 3–4 septa

Asci always 2-spored ..... *M. dispora* Stevens and Manter

Asci 8-spored ..... *M. javanica* (Rac.) Sacc. and Trott.

Spores with 3–5 septa

Amphigenous, asci at least 80 μ long

Asci 6–8-spored, spores constricted ..... *M. dissociabilis* Stevens and Manter

Asci 8-spored, spores little constricted ..... *M. evonymi* Syd.

Epiphyllous, 8-spored

Asci 40–60 μ ..... *M. orchidearum* P. Henn.

Asci 150–180 μ ..... *M. zingiberaccicola* P. Henn.

Spores 4–5, 4–5–6, or 4–6-septate

Asci 2–4-spored, usually 2, spores usually 5-septate ..*M. aroidicola* Stevens and Manter

Asci 3–4-spored, usually 4, spores usually 4-septate ..*M. guianensis* Stevens and Manter

Asci 4–8-spored, spores clavate ..... *M. rhopaloides* Syd.

Asci 8-spored ..... *M. aeruginascens* Rehm; *M. borneensis* Syd.

Spores 5–7, 5–8, or 6–7-septate

Central cell longitudinally divided ..... *M. balansae* Speg.

Spores all cross-walled only

2–8-spored, 5–8-septate, spores 50 μ ..... *M. alsodeiae* (P. Henn.) Th.

4–8-spored ..... *M. samarensis* Syd.

8-spored ..... *M. corynespora* Sacc.

Spores 8–16-celled, clavate ..... *M. congoensis* Beeli

Thyriothecia brown or brown-black[2]

Spores 5–7-septate ..... *M. leoniae* P. Henn.

Spores 3-septate

Hypophyllous, asci fusoid ..... *M. aureo-brunnea* P. Henn.

Epiphyllous, asci clavate ..... *M. rechingeri* v. Höhn.

[2] These last three species probably do not belong in *Micropeltis* because of the nature of the thyriothecia. *M. sprucei* (Cke.) Sacc. belongs in that group possessing spores with 3–5 septa, but it is insufficiently described to be given an exact position in the key.

8. **Micropeltis tetraspora** Stevens and Manter, n. sp. (fig. 30).—Thyriothecia sparse, hypophyllous, 585 μ in diameter, margin blue-green, surrounded by transparent halo; ostiole unevenly round, 40 μ in diameter; asci cylindrical to subclavate; paraphyses copious, filamentous; asci always 4-spored, 146–187×37 μ; spores 3-septate, deeply constricted, cells loosely connected, basal cell elongated; spores 93–133×13–16 μ.

On unknown host, British Guiana: Kartabo, July 24, *653*.

This fungus differs from *M. albo-ostiolata* in having 4 instead of 8-spored asci, and in greatly different spore measurements.

In general appearance and measurements it resembles *M. dissociabilis*, but this latter fungus has 6–8-spored asci and 3–4–5-septate spores.

9. **Micropeltis dispora** Stevens and Manter, n. sp. (figs. 31–33).—Thyriothecia amphigenous, 817 μ in diameter, border blue-green; ostiole round, 40–48 μ in diameter; asci cylindrical, short stipitate, 160×26 μ, always 2-spored, paraphyses copious, short, crooked, filamentous; spores 3–4–septate, very rarely 5-septate, constricted, 90–103×8–13 μ.

On unknown host, British Guiana: Kartabo, July 23, *593*.

This fungus is interesting in the constant 2-spored condition of the mature ascus, and in the variation in spore septation. Thus three asci were found in which one of the two spores was 3-septate and the other 4-septate; one ascus in which both spores were 4-septate; one ascus in which both spores were 3-septate; and one ascus was found with one spore 5-septate. The fungus differs from neighboring species (*M. evonymi*, *M. rhopaloides*, and *M. dissociabilis*) in the 2-spored condition of the ascus, none of the other species ever possessing less then six spores in the ascus. It is perhaps closer to *M. guianensis*, but this latter species is distinct from it in having 3–4-spored asci, and also in quite different spore measurements.

10. **Micropeltis dissociabilis** Stevens and Manter, n. sp. (fig. 38).—Thyriothecia scattered, amphigenous, 535–700 μ in diameter, margin blue-green, open netted; ostiole round in mature thyriothecia; opening covered with thin membrane in young thyriothecia; asci cylindrical to clavate, sessile, 6–8-spored, 160–175×38 μ; paraphyses short, crooked, filamentous; spores 3–4–5-septate, usually 4-septate, deeply constricted, cells egg-shaped when nearly mature, ellipsoidal when mature, easily separating, end cells rounded, basal cell sometimes attenuated, 87–125×12–15 μ.

On unknown host, British Guiana: Coverden, August 8, *785*.

This fungus differs from *M. rhopaloides* in shape of spores. It differs from *M. evonymi* in not having typically 3-septate spores, in number of spores in the ascus, and in having larger spores and asci; from *M. aroidicola* in size of parts and in number of spores in the ascus. The fungus is of interest in showing a striking resemblance to the common species of *Dictyothyriella*, but differing from them in having 4-septate instead of 2-septate spores. It illustrates a possible close relationship between *Micropeltis* and *Dictyothyriella*.

11. **Micropeltis aroidicola** Stevens and Manter, n. sp. (figs. 34, 35).—Thyriothecia epiphyllous, 1070 μ in diameter, margin netted, blue-green; ostiole round, 15 μ in diameter; asci clavate to cylindrical, sessile, 2–4-spored, usually 2-spored, 80–95×15–17 μ; paraphyses copious, filamentous, crooked, 1.5 times length of ascus; spores 4–6-septate, usually 5-septate, constricted, cells loosely joined, tip cell spherical, basal cell often slightly elongated; spores 30–32×10 μ.

On *Philodendron* sp., British Guiana: Kartabo, July *23*, *544*.

This fungus differs from *M. rhopaloides* and *M. borneensis* in shape of the spore and in the number of spores in the ascus. *M. acruginascens* also has a different spore shape and more spores in the ascus. *M. dispora* has a much larger spore measurement.

12. **Micropeltis guianensis** Stevens and Manter, n. sp. (fig. 36).—Thyriothecia epiphyllous, scattered, 750 μ in diameter, ostiole round, 45–50 μ in diameter; asci clavate to cylindrical, shortly stipitate, 125–140×15–25 μ, apex blunt, stipe 3–8 μ, 3–4-spored; paraphyses copious, filamentous, crooked; spores 4–5-septate, constricted, hyaline, inordinate, 50–65×10 μ.

On unknown host, British Guiana: Tumatumari, July *12*, *241*.

It differs from *M. samarensis* Syd. in having 4–5-septate instead of 6–7-septate spores, also in spore shape; from *M. rhopaloides* Syd. in spore shape and size.

13. MICROPELTIS RHOPALOIDES Syd., Ann. Myc. **15**:230, 1917 (fig. 37)

On unknown member of the Bignoniaceae, British Guiana: Penal Settlement, July *25*, *683*.

This fungus differs from the original description only in being epiphyllous instead of amphigenous, and in having slightly larger thyriothecia.

MICROPELTELLA Syd., Ann. Myc. 11:404, 1913

All *Micropeltis* species described as aparaphysate are included in the following key. EARLE described *M. longispora* as without paraphyses, but as the thyriothecial membrane is dark brown, it cannot belong to either *Micropeltis* or *Micropeltella* and is omitted from the key.

Spores with not more than 3 septa
- Ascus 5–6-spored ..... *M. exilis* (Schulz.), n. comb.
- Ascus 8-spored
  - Spores with 2–3 septa ..... *M. bogoriensis* (v. Höhn.) Syd.
  - Spores with 3 septa
    - Ascus obclavate ..... *M. minima* Stevens and Manter
    - Ascus fusoid
      - Ascus 36–40 μ ..... *M. leucoptera* (P. and S.) Syd.
      - Ascus 45–55 μ ..... *M. aequalis* (Sacc.), n. comb.
      - Ascus 85 μ ..... *M. immarginata* (Rehm), n. comb.

Spores 3–4-septate
- Ascus usually 8-spored ..... *M. consimilis* Rehm. / *M. merillii* Syd.

Spores 4-septate
- Ascus 180–200 μ ..... *M. maxima* Speg.
- Ascus 70 μ ..... *M. caunae* (Speg.), n. comb.

Spores 4–5-septate
- Spores not constricted ..... *M. acrensis* Syd.
- Spores constricted
  - Hypophyllous ..... *M. clavispora* Syd.
  - Epiphyllous ..... *M. constricta* Stevens and Manter

Spores 5–6–7-septate
- Ascus 8-spored ..... *M. agusanensis* Syd.
- Ascus 4–6–8-spored
  - Epiphyllous, spores 5–7-septate ..... *M. makilingiana* Syd.
  - Hypophyllous, spores 5–6-septate ..... *M. ramosii* Syd.

Spores 6-many septate, asci 4–8-spored ..... *M. pactensis* Syd.
Spores 7–septate, asci 8-spored ..... *M. macropelta* (P. and S.) Syd.
Spores 7-septate, asci 2–8-spored ..... *M. sparsa* Stevens and Manter
Spores 7–9-septate, asci 8-spored ..... *M. leptosphaeriodes* Speg.

M. uleana (Syd.) and *M. albo-marginata* (Speg.) Syd. are too insufficiently described to be definitely placed in the key. *M. uleana* appears to be very near *M. immarginata*, and *M. albo-marginata* seems close to *M. consimilis*.

14. **Micropeltella minima** Stevens and Manter, n. sp. (fig. 39).—Thyriothecia epiphyllous, 259–320 μ in diameter, margin

blue-green, netted, ostiole round, 20 μ in diameter; asci obclavate, flask-shaped, 8-spored, aparaphysate, 40–55×20 μ; spores 3-septate, 15–17×3–5 μ, both ends rounded, cells nearly spherical.

On unknown member of the Anonaceae, British Guiana: Tumatumari, July 9, *1002;* Kartabo, July 24, *1012;* on unknown host, British Guiana: Kartabo, July 23, *1010*.

This fungus is near *M. albo-marginata* and *M. leucoptera*. It differs in shape and size of asci, and in being constantly 3-septate. Specimen no. *1010* differed from the type species only in having larger thyriothecia (up to 468 μ). With it was associated *Dictyothyriella heterosperma*.

15. MICROPELTELLA ACRENSIS Syd., Ann. Myc. **14**:89. 1916

On unknown member of the Celastraceae, British Guiana: Rockstone, July 15, *359*.

16. **Micropeltella constricta** Stevens and Manter, n. sp. (figs. 40–42).—Thyriothecia epiphyllous, scattered, 320–450 μ in diameter, margin blue-green, only slightly carbonaceous, ostiole round, 25–35 μ in diameter; asci cylindrical to obclavate, sessile, aparaphysate, 75–88×17–25 μ, 6–8-spored; spores 3–6-septate, usually 4–5-septate, tip cell bluntly rounded, 25–45×5–10 μ, constricted.

On *Coccoloba* sp., Trinidad: Cumuto, July 16, *914;* on unknown member of the Anonaceae, British Guiana: Kartabo, July 22, *568*.

This fungus agrees with *M. acrensis* Syd. except in having plainly constricted spores. The latter specimen (no. *568*) differed from the type species in the size of the thyriothecia. Here they almost always measured about 935 μ in diameter and were mostly carbonaceous. Note should also be made of the deceptive appearance of empty asci which resemble paraphyses. Only careful examination shows that what appear to be straight paraphyses are in reality the edges of husklike, emptied, or degenerate asci. This is common in *Micropeltella*, and, in the examination of any material, structures which resemble any form of paraphyses other than the usual crooked, filamentous type, should be carefully studied.

17. **Micropeltella sparsa** Stevens and Manter, n. sp. (figs. 43, 44).—Thyriothecia epiphyllous, sparse, blue-green, only slightly carbonaceous, border membranous, transparent, thyriothecia 360–400 μ in diameter; ostiole irregularly round, 25 μ in diameter; asci sometimes visible through the thyriothecial covering, radially arranged about a non-ascigerous central region, cylindrical to fusoid, sessile, aparaphysate, thick walled, bluntly rounded at each end,

80–100×24–28 μ, 2–3–4–5–6–7–8-spored; spores fusoid-cylindrical, 6–7–8-septate, usually 7–8-septate, middle cells larger, tip cells bluntly rounded, slightly constricted, 36–48×8–10 μ.

On *Anacardium occidentale*, British Guiana: Rockstone, July 13, *1913*.

This fungus showed some points of resemblance to *M. paetensis* Syd., but differs in having much smaller thyriothecia with hyaline borders, and in number of spores in the ascus, as well as being on a widely different host.

Scolecopeltidium Stevens and Manter, n. gen.

Thyriothecia superficial, disc-shaped, true mycelium lacking, margin open netted, blue-green, ostiole round, spores filiform, many-septate, paraphyses present. Like *Scolecopeltis* except that paraphyses are present; like *Micropeltis* except that spores are filiform.

The type species of *Scolecopeltis* is aparaphysate, while about one-half of the described species possess paraphyses. In view of the fact that most of the new material encountered in the present study appeared to possess paraphyses, it has seemed convenient to separate such species by the erection of a new genus. The following former *Scolecopeltis* species fall into the new genus *Scolecopeltidium: S. salacensis* Rac., *S. gaduae* P. Henn., *S. guettardae* P. Henn., *S. trivialis* Rac., *S. bakeri* Syd., *S. connari* Syd., and *S. garciniae* Rehm.

Asci not more than 4-spored
- Asci obclavate, spores up to 48-septate . . . *S. mirabile* Stevens and Manter
- Asci cylindrical, spores 11–14-septate . *S. multiseptatum* Stevens and Manter

Asci 3–6-spored . . . . . *S. hormosporum* Stevens and Manter

Asci 8-spored
- Spores 6–7-septate . . . . . *S. connari* (Syd.), n. comb.
- Spores 6–12-septate . . . . . *S. salacensis* (Rac.), n. comb.
- Spores 10–12-septate
  - Hypophyllous, 4–8-spored . . . . . *S. bakeri* (Syd.), n. comb.
- Spores 13–16-septate
  - Epiphyllous, asci 160–175 μ . . . . . *S. liciniae* Stevens and Manter
- Spores 17-septate
  - Asci 120–130 μ . . . . . *S. garciniae* (Rehm), n. comb.
- Spores 14–20-septate . . . . . *S. gaduae* (P. Henn.), n. comb.
- Spores 15–23-septate
  - Epiphyllous . . . . . *S. costi* Stevens and Manter
- Spores 20–26-septate
  - Hypophyllous . . . . . *S. guettardae* (P. Henn.), n. comb.

This key does not include *S. trivialis*, the spore septation of which could not be found in the description. It apparently resembles *S. salacensis*, differing in an ascus measurement of 149–165×12 μ.

18. **Scolecopeltidium mirabile** Stevens and Manter, n. sp. (figs. 47–50).—Thyriothecia hypophyllous, 750–965 μ in diameter, margin wide, open netted, blue-green; ostiole round, minute, 15–20 μ in diameter; asci obclavate, sessile, 150×40 μ, 2–4-spored; paraphyses short, filamentous, not crooked; spores filiform, many-septate (up to 48), constricted, variable, sometimes curled in ascus and twice as long as ascus; 115–125×8–10 μ up to 275×8–10 μ.

On unknown member of the Simarubaceae, British Guiana: Kartabo, July 23, *678*.

19. **Scolecopeltidium multiseptatum** Stevens and Manter, n. sp. (figs. 51–53).—Thyriothecia epiphyllous, scattered, large, up to 1125 μ in diameter, ostiole round, 15 μ in diameter; asci cylindrical, sessile, broadly rounded at both ends, 125–138×27–37 μ, 2–4-spored; paraphyses filamentous, crooked; spores filiform, 12–15-septate, constricted, each cell except the two end cells slightly constricted in the middle, straight or slightly curved, 100–120×10 μ.

On Philodendron sp., British Guiana: Wismar, July 24, *1004;* July 14, *264;* Kartabo, July 22, *544*.

20. **Scolecopeltidium hormosporum** Stevens and Manter, n. sp. (figs. 45, 46).—Thyriothecia epiphyllous, 1016–1070 μ in diameter, scattered, carbonaceous, very narrow blue-green margin; ostiole irregularly round, about 25 μ in diameter, not over 35 μ in diameter; asci clavate, sessile; paraphyses filamentous, slightly crooked, branched; asci either 3 or 6-spored, 175–195×32–45 μ; spores filiform, curved, many-celled, usually 25 or 26, immature spores of 6-spored ascus in 3 rows, mature spores often curved about one another, nearly as long as ascus, 165×7–8 μ, tip cell slightly elongate, basal cell spherical and larger, other cells spherical, deeply constricted.

On unknown host, British Guiana: Kartabo, July 23, *581*.

21. **Scolecopeltidium Liciniae** Stevens and Manter, n. sp. (fig. 54).—Thyriothecia epiphyllous, 500–600 μ in diameter, ostiole round, 25–35 μ in diameter; margin of thyriothecia open netted, blue-green; asci cylindrical, sessile or very short stipitate, 160–175×45–48 μ, 8-spored; paraphyses filamentous, short, crooked, not copious;

spores filiform, 13–16-septate, not constricted, straight or slightly curved, 100×10 μ.

On *Licenia* sp., British Guiana: Rockstone, July 17, *479;* on unknown member of the Marantaceae, British Guiana: Rockstone, July 16, *423*.

22. **Scolecopeltidium costi** Stevens and Manter, n. sp. (figs. 58–60).—Thyriothecia large, epiphyllous, blue-green, netted margin, 800–900 μ in diameter; ostiole round, up to 55 μ in diameter; asci cylindrical, little or no stipe, 8-spored, 175–200 μ long when mature and 37–45 μ wide; paraphyses copious, filiform, crooked; spores filiform, many-septate, 15 up to 23, constricted, straight or curved; young spores with fewer septa, young spores spindle-shaped at stage of 4–5-septa; mature spores with middle cells larger and rounded, longest spore 137×12.5 μ.

On *Costus* sp., British Guiana: Rockstone, July 16, *425;* on *Serjania paucidentata*, British Guiana: Kartabo, July 23, *585;* on unknown host, British Guiana: Tumatumari, July 12, *234*.

Specimen no. *585* differed from the type species in being hypophyllous. While the largest spore in the type species measured 137 μ, many spores apparently mature measured about 100 μ.

SCOLECOPELTIS SPEG., Bol. Acad. Cien. Cord. 11:196. 1889

No true specimens of this genus were found in the present studies, although forms with filiform spores and with paraphyses were quite numerous. As has been noted, these have been separated from *Scolecopeltis*, which is without paraphyses, and considered in the new genus *Scolecopeltidium*. Because the two genera are so closely related, a key of the described *Scolecopeltis* species is here included. It should also be recalled that a few very similar species, differing only in possessing "stellate" ostiole, have been described by SPEGAZZINI under *Scolecopeltella*. These species are not included in the key. Following THEISSEN and SYDOW, *Scolecopeltopsis* v. Höhn. is considered as synonymous with *Scolecopeltis*.

Asci 2–3–4-spored ..... *S. aeruginea* Zimm.
Asci 8-spored
  Spores 5–7–9-septate ..... *S. transiens* (v. Höhn.) Sacc. and Trot.
  Spores many-septate
    Asci 75–100 μ (not over 100 μ)
      Hypophyllous, spores 10–15-septate
        Asci 20–25 μ wide ..... *S. pachyasca* Speg.
        Asci 42–45 μ wide ..... *S. dissimilis* Rehm

Epiphyllous
Asci clavate.....*S. bauhiniae* P. Henn.
Asci at least 110 μ long
Asci 110×40 μ, spores 40-septate.....*S. ophiospora* (Pat.) Sacc.
Asci 130–180 μ
Spores blunt, 15-septate.....*S. quindecieseptata* P. Henn.
Spores pointed.....*S. tropicalis* Speg.
Asci 180–200 μ
Spores 15–20-septate.....*S. portoricensis* Speg.

RICK has listed a species, *Scolecopeltis theissenii* Rick, under the herbarium reference "exsicc. Rick, F. a. am. 250," but no printed description of the species or reference to publication could be found.

Theciopeltis Stevens and Manter, n. gen.

Thyriothecia superficial, circular, disc-shaped, covering open netted, not radial, rich brown becoming opaque toward the center, border hyaline, no true ostiole, irregular stellate opening in ripe thyriothecia, little or no mycelium.

This genus is most like *Phragmothyriella* or *Microthyriella*, but differs quite markedly in the appearance of the covering of the thyriothecia, which is formed by a network of hyphae anastomosing or interweaving instead of the apparently cellular and easily disintegrating covering found in *Phragmothyriella* and *Microthyriella*. The breaking of the thyriothecia is at a definitely central point, while in *Phragmothyriella* and *Microthyriella* it occurs by a kind of fragmentation of the thin and quite transparent covering. On account of the netlike structure of *Theciopeltis* it is placed in the subfamily Dictyopeltineae, where it appears to be the first representative which is brown in color.

23. **Theciopeltis guianensis** Stevens and Manter, n. sp. (figs. 6, 7, 61).—Thyriothecia epiphyllous, 160–200 μ in diameter, circular, rich brown, widely open netted, border hyaline, no true ostiole, sometimes an irregular stellate opening; asci spherical (obclavate when mature), sessile, 20–30 μ, paraphyses few, slightly crooked, not branched; asci 6 or 8-spored; spores cylindrical, 1–2–3-septate, hyaline, 12–20×4–5 μ.

On unknown host, British Guiana: Rockstone, July 17, *470*.

THRAUSMATOPELTINEAE

The subfamily Thrausmatopeltineae proves somewhat more difficult, although the difficulties are chiefly connected with the species and genera. The subfamily itself is quite distinct in possessing thy-

riothecial coverings of pseudo-parenchymatic tissue brown or yellow-brown in color. The individual cells in this covering are small and tend to be angular. Often, especially when an ostiole is absent, the covering breaks down into irregular pieces (as in *Microthyriella*). Hymenia (or ascigerous tissue), according to THEISSEN and SYDOW, may be one or many in the thyriothecia, and each of these hymenia may bear one or many asci. These conditions of the hymenia are used by THEISSEN and SYDOW to separate wide groups of genera. Most forms occur in the group described as "only one hymenium present." These authors place here *Microthyriella*, although, as pictured by VON HÖHNEL (**21**), the asci in this genus are somewhat separated by hyphal growth ("pseudo-paraphyses"). Fortunately, separation of genera on the basis of the presence of "many hymenia" is rendered much less significant due to recent research. In this group, THEISSEN and SYDOW list three genera, each of but few species; *Polyclypeolum*, *Eremotheca*, and *Eremothecella*.

Eremotheca was described by THEISSEN and SYDOW in 1917 (**19**), who used *Rhytisma rufulum* B. and C. as the type, and named one new species, *E. philippinensis* Syd. In 1918, VON HÖHNEL (**26**) declared that the type species of *Eremotheca* is identical with *Microthyriella rickii* v. Höhn., and that *E. philippinensis* belongs in the same genus, differing from *M. rickii* only slightly in spore measurement. As a *Microthyriella philippinensis* had already been described, VON HÖHNEL called *E. philippinensis*, *M. macrospora*. Specimens of this fungus identified by SYDOW were available for study, and in every character seemed to agree with those of *Microthyriella*, so that we follow VON HÖHNEL in accepting *Microthyriella* as a synonym for *Eremotheca*.

Eremothecella Syd. (**19**) has but one species, *E. clamicola*. VON HÖHNEL (**27**) studied this and found it to be a lichen. It was identified by ZAHLBRUCKNER as belonging to *Arthoniopsis*. A cotype of this species was available for our study. It is evident that some lichens may be very closely similar in appearance to members of this group of the Hemisphaeriaceae. This particular specimen could very easily be mistaken as a member of the subfamily Gymnopeltineae Stevens and Guba (**11**), as there appears to be no covering over the asci.

Polyclypeolum has but a single species, *P. abietes* (v. Höhn.)

Theiss. VON HÖHNEL (**25**) expresses doubt that this genus can be separated from *Schizothyrium*.

All of the remaining genera of the Thrausmatopeltineae are more or less similar to *Microthyriella*, the most common genus. This genus was established in 1909 by VON HÖHNEL (**21**), with *M. rickii* Rehm as the type species. The covering of the thyriothecia is composed of small cells (4- or 5-sided), is brown, and lacks an ostiole. When mature the whole covering fragments, falling apart into irregular pieces. The spores are 2-celled.

Several other genera are so similar to *Microthyriella* that attention should be called to their exact position. At the time *Microthyriella* was described, VON HÖHNEL observed that it was like *Microthyrium* except that an ostiole was lacking. *Microthyrium*, however, is now considered by THEISSEN and SYDOW to belong in the Microthyriaceae, so that true *Microthyrium* species have a radial covering.

The spore septation, which is very constant here, easily separates *Haplopeltis* Theiss. (**18**) with 1-celled spores, and *Phragmothyriella* v. Höhn. (**24**) which has many celled spores. *Haplopeltis* has an ostiole, but *Phragmothyriella* is exactly similar to *Microthyriella* except for spore septation.

Clypeolum Speg. (**4**) is very similar to *Microthyriella*, as pointed out by VON HÖHNEL (**22**). The original description of *Clypeolum* gives it a carbonaceous thyriothecium and cylindrical asci, but VON HÖHNEL, in studying the type species, found the structure of the thyriothecium to agree with that of *Microthyriella*. He separated the two because of different shape of asci, and because *Clypeolum* possesses typical thready paraphyses, while the paraphyses of *Microthyriella* are branched and united into a tissue-like mass. On this basis, he allotted a number of former *Clypeolum* species to *Microthyriella*.

SPEGAZZINI, in 1923 (**8**), named a new genus, *Clypeolina*. This genus is like *Clypeolum* except that it has an ostiole. The name *Clypeolina* is preoccupied, however, as THEISSEN gave it to a genus of Microthyriaceae (**14**). We therefore propose the name *Clypeolopsis* Stevens and Manter for the genus *Clypeolina* named by SPEGAZZINI in 1923.

Schizothyrium Desm. (**2**) is also very similar to *Microthyriella*, as

shown by VON HÖHNEL (**25**). *Schizothyrium*, however, is not usually circular in form, but tends to be elongate (the type species measured 250–300 μ long by 180–200 μ wide). It has a thick, often carbonaceous membrane, instead of the thin one found in *Microthyriella*. It lacks an ostiole, but when ripe the thyriothecia open by means of a longitudinal split. The surface of the thyriothecia is sparsely covered with mycelial strands, which stream out over the angular cells of the membrane proper, and may extend slightly beyond the border.

A key to the genera of the Thrausmatopeltineae following the conclusions of this discussion follows.

One hymenium present
- Spores 1-celled, colorless ..... *Haplopeltis* Theiss.
- Spores 2-celled, colorless
  - Thyriothecia round
    - Ostiole present ..... *Clypeolopsis* Stevens and Manter
    - Ostiole lacking
      - Paraphyses present ..... *Clypeolum* Speg.
      - Paraphyses lacking ..... *Microthyriella* v. Höhn.
  - Thyriothecia elongated ..... *Schizothyrium* Desm.
- Spores many celled, colorless ..... *Phragmothyriella* v. Höhn.

More than one hymenium present ..... *Polyclypeolum* Theiss.

MICROTHYRIELLA V. HÖHN., SITZ. KAIS. ACAD. WISS. WIEN **118**:371. 1909

REHM'S former *Microphyma rickii* was taken by VON HÖHNEL as the type species for *Microthyriella*. The characters of the genus are well defined and have already been outlined. The relationships of the genus to *Clypeolum* and *Schizothyrium* have also been discussed.

Microthyriella is remarkable for the constancy of some characters which are notably variable among the Dictyopeltineae. For example, the asci are almost always ovoid-globose and sessile, always 8-spored; while the spores are constantly 1-septate. The large number of species are hence very largely separated on the size of parts. THEISSEN in 1914 (**17**) reviewed the genus up to that date, and lists fourteen species. Since then the number of species has considerably increased, and the genus is rather difficult to analyze.

Of the twenty-four species of *Microthyriella* now described, two possessed only immature spores, so their exact position cannot at

present be determined. These species are *M. celastri* (E. and K.) Th. and *M. perexigua* (Rob.) v. Höhn. To be listed as possible *Microthyriella* species are *Clypeolum sulcatum* Starb. and C. *megalosporum* Speg., both of which VON HÖHNEL (**22**) suggested as being probable *Microthyriella* species, although he did not rename them. Most of the other species which VON HÖHNEL suggested at the same time in a similar manner have since been definitely placed in the genus *Microthyriella*. In the absence of material for study, it has seemed best simply to point out that *C. sulcatum* might be near *M. uvariae* and *M. philippinensis*, although clearly distinct from them; and *C. megalosporum* might be near *M. rickii*. To these species are added two new species from British Guiana. THEISSEN (**18**) gives *M. olivacea* (v. Höhn.) Th. as a new combination for *Asterella olivacea* v. Höhn. VON HÖHNEL (**27**) later made the same change, apparently unaware that THEISSEN had already done so. The correct name therefore should read *Microthyriella olivacea* (v. Höhn.) Theiss.

As already noted, *Eremotheca philippenensis* Syd. becomes *Microthyriella macrospora* v. Höhn. *Microthyriella hibisci* Stevens and Guba (**11**) should perhaps be accorded a new genus, as the possession of an ostiole is not in agreement with the character of the genus.

Ostiole present ..... *M. hibisci* Stevens and Guba
Ostiole absent
  Spores constricted
    Asci round
      Spores 8 μ long ..... *M. intricata* (E. and M.) Th.
      Spores 37–47 μ long ..... *M. guianensis* Stevens and Manter
    Asci ovate
      Spores 10 μ long ..... *M. rimulosa* (Speg.) Th.
      Spores 28–30 μ long ..... *M. pseudoculiculosa* (Winter) v. Höhn.
  Spores not or little constricted
    Asci pyriform
      Spores 9 μ ..... *M. minutissima* (Speg.) Th.
    Asci spherical
      Asci 25 μ in diameter ..... *M. cuticulosa* (Cke.) v. Höhn.
      Asci 27–35 μ in diameter ..... *M. discoidea* (Cke.) Th.
    Asci ovoid or ellipsoid
      Spores not over 13 μ
        Asci with short stipe ..... *M. astoma* (Speg.) The

Asci sessile
Asci 16–20 μ long ..... *M. rubi* Petrak.
Asci 25 μ long ..... *M. olivacea* (v. Höhn.) Th.
Asci 50–75 μ long ..... *M. distincta* Stevens and Manter
Spores 15 μ long
Hypophyllous, spores clavate ..... *M. applanata* (Rehm) v. Höhn.
Epiphyllous, spores obclavate ..... *M. patelloides* (E. and M.) Th.
Spores 18–26 μ long ..... *M. uvariae* (P. Henn.) Th.; *M. philippinensis* Syd.
Spores at least 32 μ long
Thyriothecia 150–250 μ, epiphyllous ..... *M. uleana* Syd.
Thyriothecia at least 500 μ
Asci 120–140 μ ..... *M. malacoderma* v. Höhn.
Asci 90 μ ..... *M. longispora* (Pat.) Th.
Asci not over 85 μ
Spores elongate, 60–70 μ ..... *M. coffeae* (P. Henn.) Th.
Spores oblong, not over 55 μ
Asci elliptical ..... *M. rufula* (B. and C.) Th. and Syd.
Asci ovate
Spores 32–36 μ ..... *M. rickii* (Rehm) v. Höhn.
Spores 36–44 μ ..... *M. modensis* (P. Henn.) Th.; *M. scutelliforme* (Rehm) Th.
Spores 42–55 μ ..... *M. macrospora* v. Höhn.

24. MICROTHYRIELLA INTRICATA (E. and M.) Theiss., Ann. Myc. 10:190. 1912

On *Tapira* sp., British Guiana: Kartabo, July 22, *980*.

25. **Microthyriella guianensis** Stevens and Manter, n. sp. (fig. 62).—Thyriothecia hypophyllous, brown, tissue psuedo-parenchymatous, very delicate and easily breaking into parts, flat, non-carbonaceous, no ostioles, 375–535 μ in diameter; each ascus in separate locule, the locules separated by a thready tissue, asci spherical, 50–65 μ in diameter, aparaphysate, 8-spored; spores 2-celled, hyaline, constricted, cells equal, spores 37–47 × 10–12 μ.

On unknown member of the Ochnaceae, British Guiana: Tumatumari, July 10, *130*.

This fungus differs from *M. macrospora* in having spherical asci and in the constricted spores. It differs from *M. rufula* in the same respects, and from *M. intricata* by possessing much larger parts.

26. **Microthyriella distincta** Stevens and Manter, n. sp. (fig. 63).—Thyriothecia epiphyllous, gregarious, entirely flat, up to 1.5 mm. in diameter, brown, non-carbonaceous, not always circular but

often irregular in outline, covering membrane pseudo-parenchymatous, hymenium single, no ostiole; asci many, elongate, subcylindrical, ellipsoid, blunt at both ends, aparaphysate, 8-spored, 50–75 × 20–25 μ; spores colorless, 2-celled, ellipsoid, not or little constricted, not parallel, 12–13 × 5–6 μ.

On unknown host, British Guiana: Kartabo, July 24, *675*.

The spores of this species are very small in relation to the size of the ascus. This proportion separates it from all other species in the genus.

PLOCHMOPELTINEAE

The Plochmopeltineae Theiss. (**18**) are separated from the other subfamilies of the Hemisphaeriaceae by possessing thyriothecial membranes of meandering, sinuous context, and also by possessing a free mycelium without hyphopodia. The thyriothecia are brown, although this character is not given in the subfamily description. Only a few genera are known. No representatives with mature spores were found in the present studies. MENDOZA, however, working at the University of Illinois, has described two new genera and a few new species in this group. These genera and species are included here, although a part of the material was collected in Hawaii. The descriptions of these forms are as given by MENDOZA, and the key to the genera is modified by him from THEISSEN and SYDOW.

Covering membrane without ostiole, paraphyses lacking
- Spores 2-celled, hyaline
  - Covering membrane without setae ..... *Plochmopeltis* Theiss.
  - Covering membrane with setae ..... *Plochmopeltidella* Mendoza

Covering membrane ostiolate, hymenium with many asci
- Spores 2-celled, hyaline
  - Covering membrane without setae
    - Paraphyses present ..... *Stomiopeltis* Theiss.
    - Paraphyses absent ..... *Stomiopeltella* Theiss.
  - Covering membrane with setae ..... *Chaetopeltopsis* Theiss.
- Spores 4-celled, hyaline
  - Covering membrane without setae
    - Paraphyses present ..... *Scolecopeltidella* Mendoza

Plochmopeltidella Mendoza, n. gen.

Mycelium netlike, interwoven, with long cylindrical cells; thyriothecia without ostiole, covering membrane a winding prosenchyma, setose, with many asci, aparaphysate; spores hyaline, 1-septate.

27. **Plochmopeltidella smilacina** Mendoza, n. sp. (figs. 64–66).—Mycelium of a netlike weft, interwoven, with long cylindrical cells, straw colored to almost brown, branching, without hyphopodia; thyriothecia covered by a winding prosenchyma, without ostiole, setose, about 120 μ in diameter; setae few, acute, black, about 130 μ long and 9 μ wide near base, borne on thyriothecia and outgrowths from the long cylindrical cells; asci numerous, sickle-shaped to clavate, 8-spored, about 36×12 μ; spores more or less fusiform, hyaline, 1-septate, about 12×4 μ.

On *Smilax* sp., Oahu: Olympus, June 6, *670*.

The characters of this fungus show it to be nearly related to *Plochmopeltis* Theiss., except in the presence of setae, which are not found in *Plochmopeltis*. Such difference is sufficient to warrant the erection of a new genus. The fungus is also associated with several other fungi, among them *Phragmocapnias smilacina*.

STOMIOPELTIS THEISS., Broteria **12**:82. 1914

28. **Stomiopeltis cassiae** Mendoza, n. sp. (figs. 67, 68).—Mycelium irregularly branched, woven to weftlike, hyaline to honey-yellow; thyriothecia numerous, dimidiate, covering cells long, irregularly woven, yellowish to brown, ostiolate, about 100 μ in diameter; ostiole with definite border, about 7 μ in diameter; asci numerous, ovate, aparaphysate, 8-spored, about 36×9 μ; spores ovate, hyaline, 1-septate, about 13×3 μ.

On *Cassia* sp., British Guiana: Tumatumari, July 10, *115*.

This fungus is found in pure growth on the leaf, and appears as a leathery, thin, sooty mass on the upper surface. The colonies have no definite shape, and are more or less irregularly scattered, although they sometimes cover the whole surface. It is strictly epiphyllous.

Stomiopeltis aspersa (Berk.) Theiss., formerly called *Asterina aspersa*, and *Stomiopeltis rubi* (Fuck.) Petr., formerly called *Asterella rubi* (Fuck.) v. Höhn., are the only other species in this genus.

STOMIOPELTELLA THEISS., Broteria **12**:85. 1914

29. **Stomiopeltella suttoniae** Mendoza, n. sp. (figs. 69–72).—Mycelium irregularly branched, gelatinous, honey-yellow to dark brown; thyriothecia numerous, dimidiate, covering cells long, irregularly woven and interwoven, with long cylindrical cells, yellowish to dark brown, ostiolate, gelatinous, about 120 μ in diameter; asci nu-

merous, ovate, aparaphysate, 8-spored, about 44×16 μ; spores ovate, hyaline, 1-septate, about 14×5 μ.

On *Suttonia lessertiana*, Oahu: Honolulu, Hamakua upper ditch trail, July 28, *1032*.

This fungus appears to agree fully with *Stomiopeltella*, of which *S. patagonica* (Speg.) Theiss. (formerly called *Microthyrium patagonica*) is the type species. It differs from the type species in having ovate instead of elongate asci and in wider spores. It differs from *S. nubecula* (B. and C.) Theiss. (the only other species in the genus) by having much smaller asci, which are ovate instead of cylindrical.

Scolecopeltidella Mendoza, n. gen.

Mycelium netlike, branching, hyaline; thyriothecia hemispherical, membranous, ostiolate, honey-yellow; asci 8-spored, paraphysate; spores hyaline, cylindrical, 3-septate.

30. **Scolecopeltidella palmarum** Mendoza, n. sp. (fig. 73–75).—Mycelium netlike, irregularly branched, hyaline to very pale straw-colored, radiating irregularly from the thyriothecia; thyriothecia membranous, honey-yellow, ostiolate, with indefinite border, about 168 μ in diameter; asci numerous, ovate, paraphysate, 8-spored, about 36×12 μ; paraphyses numerous, thready, spores hyaline, long-cylindrical, 3-septate, about 19×4 μ.

On palm, British Guiana: Rockstone, July 17, *442*.

This fungus resembles *Scolecopeltis* in the general shape of the thyriothecia, the presence of an ostiole, and the shape and septation of the spores. It differs from *Scolecopeltis* in that it has paraphyses, 4-celled spores, a true superficial mycelium, and honey-yellow color. *Scolecopeltis* has many celled spores, blue-green thyriothecia, no true mycelium, and no paraphyses. The fungus appears as yellowish to almost brown specks, thinly scattered on both surfaces of the leaf. The thyriothecia are rare.

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EXPLANATION OF PLATES XVIII–XXI

Plate IV was copied from figures by Mr. JOSE MENDOZA; all other drawings were made by the aid of a camera lucida by MR. MANTER.

PLATE XVIII

FIG 1.—Leaf showing characteristic appearance of thyriothecia of fungus (*Micropeltella constricta*).

FIG. 2.—Enlarged portion of same.

FIG. 3.—Thyriothecia of *Dictyothyriella vismiae* on *Coccoloba* leaf.

FIG. 4.—General appearance of thyriothecia of Dictyopeltineae.

FIG. 5.—Portion of border of same enlarged to show netlike structure.

FIG. 6.—Thyriothecium of *Theciopeltis guianensis*.

FIG. 7.—Portion of border of same.

PLATE XIX

FIG. 8.—Asci and ascospores of *Dictyothyrium disporum*.

FIG. 9.—Asci and ascospores of *Dictyothyrium leucopterum*.

FIGS. 10–13.—*Dictyothyriella guianensis;* asci and ascospores, showing variation.

FIGS. 14–17.—Asci and ascospores of *Dictyothyriella heterosperma*, showing variation in number of spores.

FIGS. 18–22.—Asci and ascospores of *Dictyothyriella heterosperma* from single perithecium, showing great variability of spores.

FIG. 23.—Asci and ascospores of *Dictyothyriella heterosperma* from different host.

FIGS. 24–26.—Asci and ascospores of *Dictyothyriella philodendri*.

FIGS. 27–29.—*Dictyothyriella vismiae*; asci and ascospores.

FIG. 30.—Asci and ascospores of *Micropeltis tetraspora*.

FIG. 31.—Ascus, ascospores, and paraphyses of *Micropeltis dispora*.

FIG. 32.—Ascospores of same.

FIG. 33.—Ascus of same with spores.

FIGS. 34, 35.—Asci and ascospores of *Micropeltis aroidicola*.

FIG. 36.—*Micropeltis guianensis;* asci, paraphyses, and ascospores.

PLATE XX

FIG. 37.—Ascus and spores of *Micropeltis rhopaloides*.

FIG. 38.—Asci and spores of *Micropeltis dissociabilis*.

FIG. 39.—Asci and spores of *Micropeltella minima*.

FIG. 40.—Ascus and spores of *Micropeltella constricta*.

FIGS. 41, 42.—Asci and spores of same fungus from different host.

FIGS. 43, 44.—Asci and spores of *Micropeltella sparsa*.

FIGS. 45, 46.—Asci of *Scolecopeltidium hormosporum*, showing ascospores.

FIGS. 47–49.—Spores of *Scolecopeltidium mirabile*.

FIG. 50.—Spores of same in portion of ascus, showing position within ascus.

FIGS. 51–53.—Asci and spores of *Scolecopeltidium multiseptatum*.

FIG. 54.—Ascus and spores of *Scolecopeltidium liciniae*.

FIGS. 55–57.—Outline of ostioles of different thyriothecia on same leaf, showing variation (*Dictyothyriella vismiae*).

FIG. 58.—Asci of *Scolecopeltidium costi*, showing spores in very early stage of development.

FIG. 59.—Asci of same fungus showing spores at different stages of development.

FIG. 60.—Mature spores of *Scolecopeltidium costi*.

FIG. 61.—Ascospores of *Theciopeltis guianensis.*

FIG. 62.—Asci and spores of *Microthyriella guianensis.*

FIG. 63.—Asci and spores of *Microthyriella distincta.*

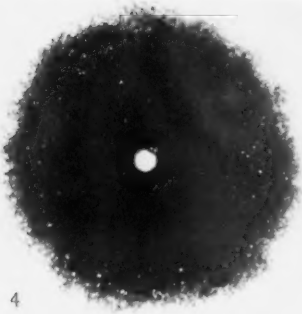
PLATE XXI

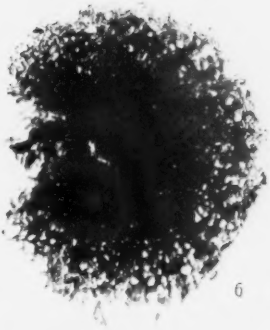
FIGS. 64–66.—*Plochmopeltidella smilacina:* fig. 64, thyriothecium showing character of covering membrane and portion of setae; fig. 65, two asci with ascospores; fig. 66, three ascospores showing sickle-shape to clavate form.

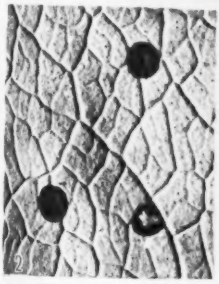
FIGS. 67–68.—*Stomiopeltis cassiae:* fig. 67, thyriothecium showing ostiole and asci with ascospores; fig. 68, ascus with ascospores.

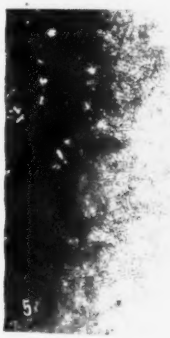
FIGS. 69–72.—*Stomiopeltella suttoniae:* fig. 69, portion of mycelium showing character of cells; fig. 70, thyriothecium showing ostiole and character of covering membrane; fig. 71, ascus with ascospores and paraphysis; fig. 72, two ascospores.

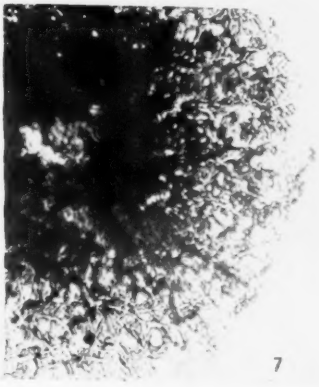
FIGS. 73–75.—*Scolecopeltidella palmarum:* fig. 73, thyriothecium showing ostiole and character of covering membrane and cells of mycelium; fig. 74, ascus with ascospores and paraphyses; fig. 75, two ascospores.



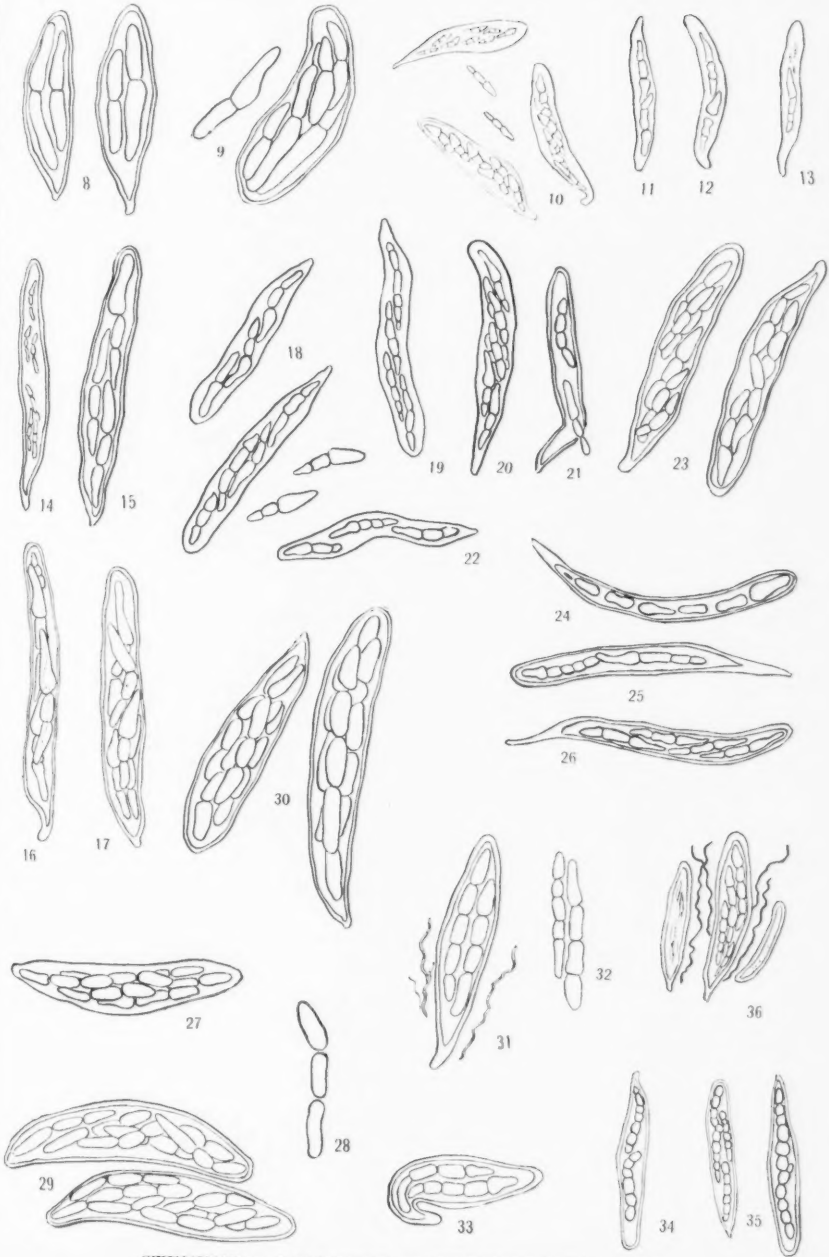








STEVENS and MANTER on HEMISPHAERIACEAE



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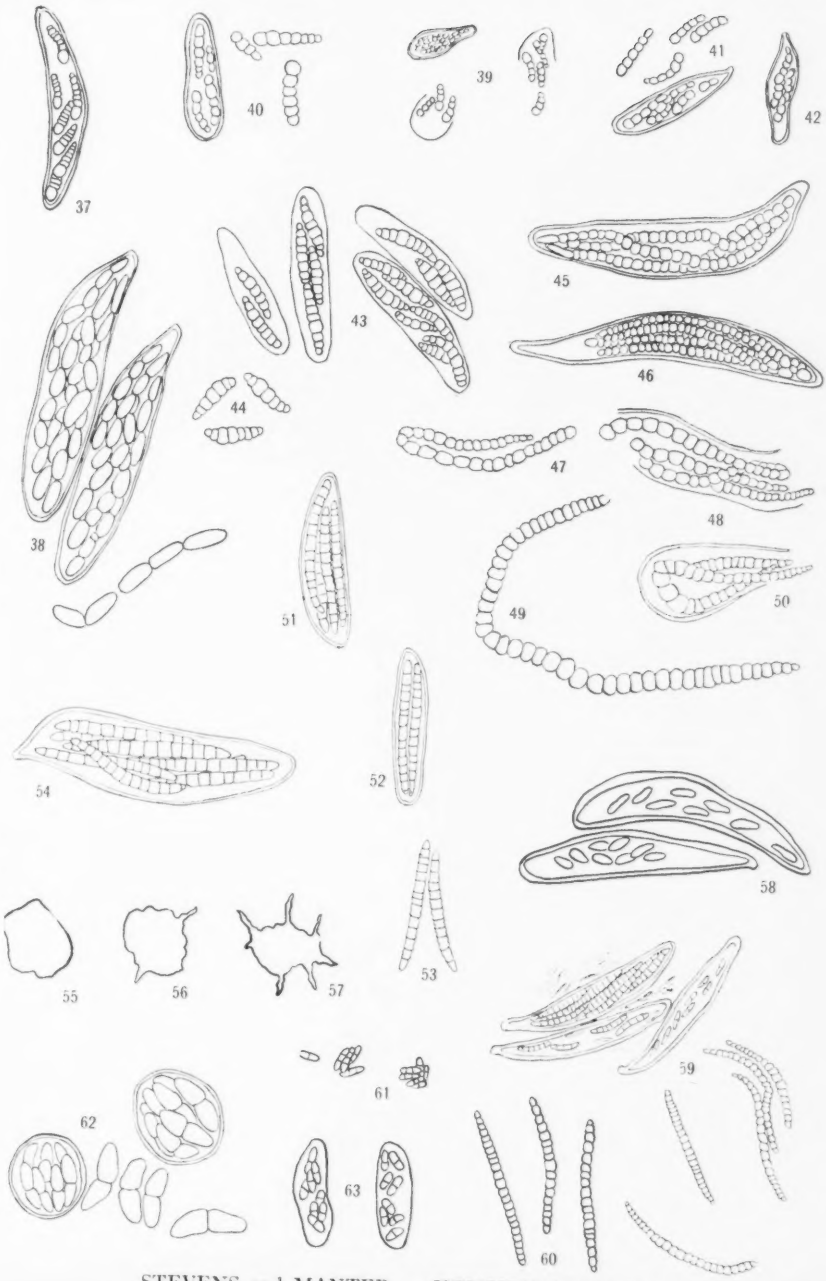

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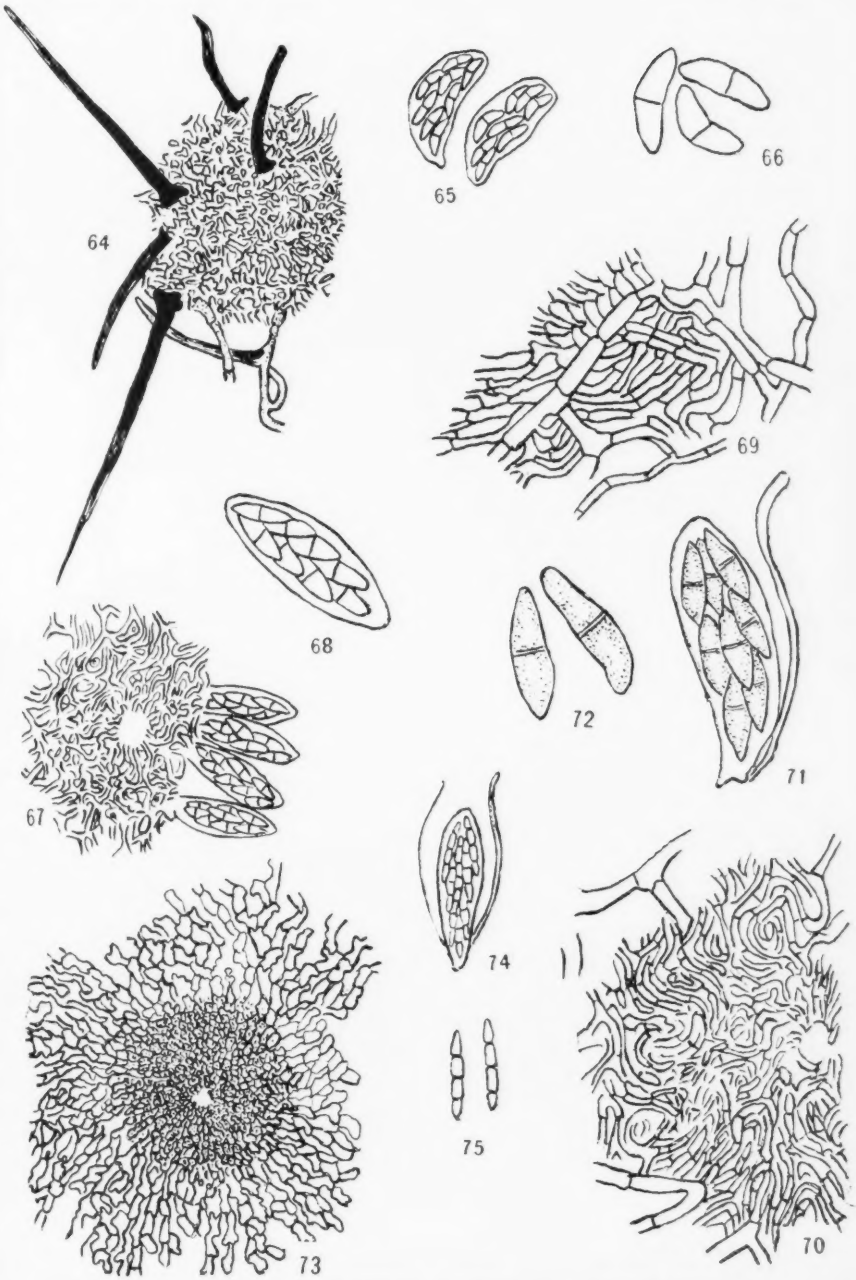

STEVENS and MANTER on HEMISPHAERIACEAE

ONTOGENETIC STUDY OF PHYLLOXERA CARYAESEPTEM

A. C. MARTIN

(WITH PLATES XXII–XXV)

In the prosecution of this study the primary objective was an investigation of the developmental phases of *Phylloxera caryaeseptem* Shimer. This gall fulfills the qualifications which KUSTER (4) considered essential to "prosoplasmas," in that it possesses distinct and definite size, shape, and internal organization; tissue differentiation is not extensive but shows striking fundamental contrast to the normal leaf histology. The gall is a heteroplastic hyperplasia of the "walled" or "umwallungen" type, and in its occurrence it is restricted to the leaves of hickories. In his synoptical table to *Phylloxera* galls occurring on *Carya*, PERGANDE (5) establishes four major units, and *P. caryaeseptem* is placed with eleven other galls in group 1.

Historical

Apparently COOK (3) is the only one who has made any previous recorded histological study of *Phylloxera* galls on hickories. He examined several species, but the information which he contributed is very scant. The failure of European cecidologists to conduct research on this group is doubtless accounted for by the fact that the common hickories are restricted in range to eastern North America. So far as could be determined, the histology and morphogenesis of *P. caryaeseptem* has not been studied before.

Technique

Specimens for microscopic study were collected in the vicinity of Raleigh during April, 1922. Material was killed and fixed in the weaker solution of Flemming's fluid, or in stock chromo-acetic solution, then imbedded in paraffin, sectioned on a microtome, and stained with the safranin-light green combination. Very early stages were secured for study by close observation of a particular tree which had been badly infested with this species of gall in the pre-

ceding year. Drawings were made with the aid of a camera lucida and with a home-made projector.

Description of gall

In "Group I" the galls are "thin, paper-like, more or less transparent." PERGANDE'S description of the mature gall of *P. caryaeseptem* is in part:

> Their transverse diameter at the plane of the leaf ranges from 5 to 12 mm., and their vertical diameter from 4 to 6 mm. They are quite convex on both sides, and generally more conical and more prominently projecting above than beneath, especially so in the smaller specimens. All are provided with a nipple on both sides, the upper one stouter and more prominent. . . . . Both nipples, particularly the lower one, lean frequently somewhat toward one side. Both openings are either round or oval and fringed by eight stout or slender filaments. The opening on the under side closes gradually, so that the insects are compelled to leave from above. The cavity, if cut vertically, appears to be more or less hexagonal at the central portion, more or less pointed toward the nipples, and quite flat at the median circumference, with the exception of a ridge which indicates the former division of the gall into two compartments while young.

The galls occur either isolated or aggregated, sometimes twenty-five or more on a single leaflet. When numerous they incline to fall below the normal size, indicating the probability that there is competition for a limited food supply. Frequently the bases of the galls are confluent, but in this event the chambers of the different galls do not merge. The characteristic location is in close relation to fairly prominent veins, but some specimens were found in which the vein relationship was not in evidence. Most of the specimens collected were a fairly bright green with or without a tinge of red near the base. The filaments on the nipples fold back conspicuously at maturity. The species is common near Raleigh, and certain local hickory trees are subject to severe and recurring annual attacks.

The temporary transverse partition to which PERGANDE makes reference, and from which the gall and gall insect derived their names, has been noted by cecidologists, but the nature and significance of this structure seem to have escaped detailed investigation. Interest in the morphogenesis of the septum served as the chief incentive for the present study.

Normal leaf

When the aphids commence activity the leaflets are about 0.065 mm. in vertical dimension, whereas at maturity the thickness is about 0.14 mm., fully twice as great (figs. 6, 7). At first the tissues are compact, but later air spaces appear, particularly in the lower mesophyll. Included within the upper and lower epidermal layers are three primitive mesophyll layers, the palisade layer and two other distinct layers below it. The central layer is relatively clear, whereas the lower one contains much granular material, and the cells of the palisade layer are spotted with chloroplasts and other matter. Perceptible vertical lengthening of the palisade cells is evident at a very early stage, and this tissue remains as a single distinct layer during the natural life of the leaf. With increasing development of air spaces in the mesophyll in advancing maturity, the two rows below the palisade layer lose their stratified identity and become organized into typical spongy parenchyma. Even before this modification in arrangement of cells, while the leaf is still very young, there tends to be some cell divison in the plane of the leaf, so that the layers are not uniformly one cell in thickness. The three inner layers, however, are quite distinct at the time of gall inception.

The upper leaf surface is nearly glabrous, but the lower is thickly beset with stellate hairs and with peltate scales of two sizes (fig. 2). Exceedingly long, septate, unbranched hairs are present on the lower surface of the larger veins.

Aphid

The nymph (fig. 15) is about 0.1 mm. long and about 0.06 mm. wide. With the exception of the dark legs and the reddish eyes, it is of a bright orange-yellow color. The proboscis is very long and extends some distance behind the body. Numerous progeny of the stem mother appear in the gall shortly after the disappearance of the septum. The aphid operates on the under side of the leaf almost exclusively, and this surface is so tomentose that the insect stays aloft in the stellate trichomes during the juvenile phases of the gall, and does not come into direct contact with the leaf epidermis except as it inserts its setae. The length of the aphid's proboscis appears to be an evolutionary adjustment to the hirsute character

of the lower leaf surface, the insect's response to defensive specialization on the part of the leaf.

It might be assumed that the presence of the aphids on the lower surface, and their absence on the upper, is due to the fact that the lower surface is the first to be exposed when the folded leaf extends from the opening bud, but the correct explanation probably lies in the adaptability of certain characteristics of the lower side to the requirements of the gall maker. In the examination of prepared slides, and also in macroscopic inspection of young, living galls, instances were noted in which aphids were present on both sides of the septum of the same gall (fig. 14). These instances were exceptional and therefore do not help to explain the two-chambered character of the gall. Normally only one chamber, the lower, is inhabited, and the typical development of the whole gall is controlled by one aphid. In the cases where two aphids were found living in the same young gall, it appears that proliferation was initiated by the insect on the lower surface of the leaflet, and that the other aphid, while moving over the upper surface, chanced on the empty upper compartment and made itself at home.

Very young aphids in rudimentary galls were observed in the execution of a regular periodic contraction and extension of the abdomen. In contraction, the abdomen was reduced to about one-half its normal length and was curved downward slightly. The contraction was maintained for a second or two and then released gradually. In the particular individuals studied the movement was seen repeated at intervals of about 40 seconds. The insects escape through the upper nipple four or five weeks after the stem mother begins gall formation.

Gall development

Macroscopic

The first superficial evidence of gall formation is the appearance of a small, circular, translucent area in which the aphid can be discerned. This area increases in size, and at a slight distance beyond its rim a pink or red border develops, and usually remains as a permanent character of the gall. Incident to the growth of conical walls on both sides of the leaf (figs. 1–3), the transparent area is

gradually obscured and finally hidden entirely. While the gall is still quite small, the separate chambers in it are united into one large cavity (fig. 5) by the breaking of the intervening wall. The gall increases in horizontal dimension very rapidly, and as it does so its chamber and the surface walls become extended horizontally.

Microscopic

Septum.—In the early embryonic condition of the gall, the septum shows much of the typical character of the normal young leaf. Here as elsewhere there are the usual five layers, the columnar palisade cells, and the relatively clear and transparent central layer. Gradually the normal features disappear, and although the process is much less pronounced in the septum than in other parts of the embryonic gall, there is seen a tendency toward the development of a homogeneity of tissue, which Wells (7) has interpreted as a recapitulative phenomenon characteristic of gall morphogenesis. This tendency was noticed in the palisade tissue on the loss of two normal characteristics; the chloroplasts become obscure and the typical shape disappears. In the palisade layer and the central layer the cells are larger than others in the septum, and they incline to broaden horizontally until many of them have a somewhat cubical shape (fig. 11). The epidermal layers are modified only slightly, but even in these some enlargement and horizontal elongation are evident. The tissues remain compact; no air spaces appear. All of the three mesophyll layers exhibit conspicuous hypertrophy, and the constituent cells seem to have an excessive protoplasmic content. This portion of the septum appears to be relatively quiescent meristem held in protracted dormancy.

Limited hypertrophy and horizontal elongation are not the only expressions of growth which occur in the cells of the septum. Although it is customary for this region to exhibit a minimum of hyperplasia as contrasted with the rest of the gall, some cell division does take place. This is in either one of two ways, vertically or horizontally. Vertical division, that is, division in a plane at right angles to the plane of the leaflet, occurs very sparingly in the epidermal layers, but it is not infrequent in the mesophyll, especially in the lower layer. Horizontal division in the upper epidermis of the

septum (fig. 11), causing subdivision of this layer into several rows, was noted several times and is not rare, yet it appears to be atypical, and, as will be explained below, it seems somewhat abortive. The lower epidermis, in the region of the septum, is the most static part of the entire gall; aside from very restricted hypertrophy no conspicuous growth development occurs in it. In time, all of the cells of the septum tend to flatten out laterally as the gall grows (fig. 12). The mesophyll cells carry this tendency to the extreme of attenuation, and gradually they disintegrate and collapse, leaving only the two united epidermal layers as a barrier between the upper and lower chambers (fig. 16). These changes in the septum are noteworthy, and their significance will be treated in an etiological consideration. Ultimately the epidermal layers capitulate, and the fragile barrier is broken in one or several places.

CHAMBER WALLS.—In the region bordering on the septum very active meristem develops, and here there is a rapid and pronounced approximation of homogeneity. In cross-sections of young galls, there is evident a distinct transition from the five layers of the septum to the multi-ranked condition which is attendant on active proliferation in the meristematic zone at the base of the walls. The proportionate amount of hyperplasia accruing in each of the original five layers can be determined by noticing the size and arrangement of the component cells. The mesophyll layers all divide extensively and do so approximately uniformly. The epidermal layers also divide, but generally the lower epidermis proliferates more slowly than the upper epidermis. Possibly there is some relation between this differential growth and the variance in direction of the first protrusion of the upper and lower chamber wall. The sides of the upper chamber begin to converge very early, whereas the walls of the lower chamber usually diverge at first (figs. 13, 14). In time, pronounced convergence manifests itself on both sides, and in the mature gall the walls form horizontal or gently sloping roofs, from which the chimney-like nipples project vertically (fig. 17*a*).

Ordinarily the walling-up process commences almost simultaneously on both sides of the leaflet, but in certain cases, already alluded to, horizontal division was found in the upper epidermis of the septum, and it was noted that there was a correlated tardiness in

the early growth of the upper cone (fig. 11). Evidence, however, indicates that even in perfectly normal galls the lower walls tend to elongate more rapidly than do the upper ones.

Practically all of the cell division in the wall region is horizontal; the cells are pushed out in vertical rows and elongate in the direction of wall development. Enormous hypertrophy appears in some parts of the gall. Fig. 14 gives the outline of a single giant cell, and included within its walls is shown normal leaf tissue in cross-section drawn to the same scale of magnification as the hypertrophied cell. The cells in all but the lowest and outermost portions of the walls are all large. Those in the nipples are much elongated. The smallest cells are found in line with the plane of the old septum, and also near the basal rim of the gall. All the cells, whether relatively large or small, are compact; no air spaces develop.

A nutritive zone develops as an inner lining of the gall chamber. This takes place after the septum has disappeared. The cells in this zone contain much dark colored material. The cell walls are thickened and appear to have gelatinized middle lamellae; no lignification is evident. Short, simple, septate hairs with swollen bases are numerous along the inner surface layer of this tissue. The entire zone consists of only a few layers of cells in the ceiling and floor of the gall, but at the sides of the chamber it covers a very extensive area. The first formation of nutritive tissue is in the cells contiguous to the septum region, and it is here that the cell walls attain a maximum of swelling. Immediately beyond the nutritive area the cells are essentially homogeneous, even though there is a wide range in size and shape. The cell walls are thin, and within the cells much stored starch is present. In the part of the gall adjoining normal leaf tissue, the part farthest removed from the gall chamber, dedifferentiation is incomplete. Vertical rows of cells containing dark material were frequent, and these rows appear to be large editions of similar ones found in the leaf. Another resemblance to leaf tissue is in the relative clearness of the cells representing the central layer.

A reticulated system of conductive vessels ramifies through the main part of the gall, and small bundles are found extending part way into the nipples. Hyperplasia seems to be particularly pronounced in veins which are included in the gall. An epidermis is

developed on the outside of the gall, but there is no distinct protective layer in the nipple region. This suggests an explanation of the premature drying and curling back of the nipple filaments. There is copious growth of unbranched, septate hairs of variable length on the inner side of the nipple. These project in the direction of the nipple mouth. The septate type of hair was noted nowhere else on the hickory leaf except on the largest veins. There is a scattering of stellate or simple hairs over the basal surfaces of the gall.

Starch grains

Very large grains, which give a very definite starch-iodine reaction, are found abundantly in many of the cells outside of the nutritive region. Some of the largest of the simple type attain a diameter of 20 μ. The size of the bodies varies greatly within one gall (figs. 8, 10); the smallest are found near the basal margin of the gall and the largest are produced near the corners of the mature chamber (as seen in vertical section). There is a fairly regular gradation in size of grains in the area intermediate between these two regions. The grains in the upper half of the gall are distinctly larger, more numerous, and stain more brightly than those in the lower part. Many of the bodies are found in stages of disintegration, and throughout the gall cells and groups of cells are encountered having nothing but fragmentary skeletons of these structures. Beside the nutritive tissue and epidermis, the nipples and the lower regions of the gall are usually devoid of grains. Both simple and compound grains are found. The simple forms are spherical in shape. The compound ones are of two types. Partitions extend either a short distance or fully across the body; both conditions are obtained, so according to HABERLANDT (**2**) some of the starch grains are partially compound and others are completely compound. Concentric layering was obscurely shown in some specimens. The presence or absence of amyloplasts was not conclusively determined.

Three other *Phylloxera* galls on the hickory were examined for starch grains. *P. caryaecaulis* Fitch and *P. caryaeglobuli* Walsh showed numerous enlarged grains. In *P. caryaecaulis* many of the cells were literally stuffed with starch (fig. 9). On the other hand, no starch grains were distinguished in *P. caryaevenae* Fitch.

Nuclei

Appreciable enlargement of the nucleus and its nucleolus is observable in most cells of the young gall. According to measurements, both the nucleus and the nucleolus are about a third larger than normal. In cells which appeared especially vigorous, there was a more or less extensive clear area between the nucleus and the nucleolus; in contrast, other cells showed constriction of the nuclear membrane, dim nucleoli, and much granular matter in and about the nucleus. The latter symptoms were found often in cells of the lower epidermis of the septum, and also appeared in many aging cells in other parts of the gall. In the cells of the juvenile stages the nuclei are suspended near the center of the protoplast. HORSFALL (3) calls attention to a tendency of nuclei to orient themselves within the cell toward the side near a puncture path made by the insect. Particular examination on this point in *P. caryaeseptem* gave no confirmation of HORSFALL's observation. The only recorded tendency toward a particular orientation was in the upper epidermis of the septum. Here the nuclei are frequently located on the lower walls of the cells. Normal mitotic figures were found in a number of nuclei.

Aphid punctures

The cells in and adjacent to a wound normally suffer partial collapse. This tendency facilitates the detection of the course taken by the piercing mouth parts of the insect, and makes it possible to discover the location of different wounds. Apparently the very first puncture is indistinguishable from others; its location was not determined. Very early insertions of the setae were noticed in the tissues which give rise to the chamber walls, and it may be that the filaments which constitute the terminus of the nipple have their origin in definite relation to these punctures. Almost invariably punctures were made in the tissues just beyond the point of origin of the walls. Very frequently large veins bound part of the gall, and in such cases puncture channels appear in the vein, and also in the tissues immediately beyond. In that part of the gall circumference where a vein is not present, a slight contraction of the leaf thickness was perceived near marginal punctures. Most of the early wounds made

by the insect traverse the entire thickness of the leaf. Possibly this fact is related to the double chambered character of the gall.

The paths of the setae are partly intracellular, but are mostly intercellular. They appear to follow the course of least resistance through the middle lamellae, and take short cuts through the cells only occasionally. Here, as in HORSFALL'S studies, side branching from the original channel was found. This seemed to indicate that the insect had partly withdrawn its setae and had then plunged them into fresh tissue. Enveloping sheaths which developed around the setae, and which extend outward a variable distance from the end of the channel, were common. A few instances were noted in which a sheath was formed on the upper side of the leaflet, on the opposite end of the channel from that at which the puncture was made. While the septum is intact it serves as the favorite feeding location of the aphid. Even when nothing but the epidermal layers endures as evidences of the original leaf tissue in the septum, the insect continues to suck through the remaining thin structure. After the septum breaks, the aphid still works on the rim of the septum or probes into closely adjoining tissue.

In regard to response from setal insertion, and also in regard to number of punctures, my observations conflict with those of ROSEN (6), who made a study of the development of the gall built by *P. vastatrix* (*P. vitaefoliae*). ROSEN conducted experiments in which he produced galls artificially by the use of chemicals. He states:

> It will be seen that in the intumescences produced by the application of chemicals, the place of application is the place of excessive growth, and in the *Phylloxera* the place of application is the place of hindrance of growth. From these experiments, the burden of proof becomes more difficult for those who adopt the "chemical" theory of gall production for sucking insects.

In the present study of *P. caryaeseptem*, the setal paths were found not only in the parts where growth is slow or lacking, but they also occur conspicuously in the regions of rapid proliferation. Even disregarding this fact, it seems to the writer no more difficult to explain the inhibition of growth in the locality of application of an "initial" stimulus on a chemical basis, than on a mechanical or physical one. In the case of *P. vastatrix*, ROSEN concluded that the insect made but one leaf puncture, and that in this puncture the setae remained

more or less permanently fixed. Similar habits have been noted in certain other gall makers, by other investigators. In *P. caryaeseptem*, however, the punctures are numerous. In speaking of the apparent immobility of the insect in the gall he studied, ROSEN states that "A proof of this (immobility of the insect) is the frequency with which I have found the proboscis in the tissues in many of my prepared slides." The same tendency for the setae to break off and remain in the puncture was observed very often in *P. caryaeseptem*, but in this gall it was clearly evident that loss of the setae did not indicate immobility of the aphid.

Etiology

Although it appears to the writer that tension, and such physical changes as are wrought by the sucking of the aphid, should not be considered as the primary factors in gall formation, still these phenomena may play a conspicuous rôle in determining certain characteristics of the gall, and therefore they are mentioned in an etiological connection in this paper. Evidence which indicates that there is exertion of tension on the septum is deduced from certain features of gall development. Cells of the septum which at first have their long axis in a vertical direction, later become elongated horizontally. Furthermore, measurements show that from the period of gall incipiency until the stage when the septum breaks, the horizontal diameter of the gall cavity is either doubled or trebled. By the time the gall is nearly mature, the diameter has increased 5–12 times. It seems logical to assume that this growth in width of the gall cavity tends to stretch the septum horizontally. While it seems obvious that tension is a factor in transforming the septum, however, it might be maintained that the sucking of the insect acts concurrently with tension, and wields considerable control over the alterations which take place in this part of the gall. Support for this inference might be cited in the collapse and disappearance of the mesophyll cells previous to the disorganization of the epidermal tissues. If growth tension is considered the only influence active in changing this part of the gall, it may appear difficult to reconcile the sole mechanism of this force with the premature disappearance of the mesophyll layers. For those who discount the concept which

ascribes etiological importance to the bodily removal of material from the leaf by the aphid, the logical recourse seems to lie in the search for peculiarities of the epidermis, which would account for the comparative tenacity of this tissue. Mere thickness and strength of cell walls do not explain why the epidermis persists while the mesophyll cells elongate and collapse. There is one feature of the epidermis, however, which tends to render the tension theory self-sufficient and make the suction explanation superfluous. It is the fact that vertical cell division occurs in the epidermis while the septum is being stretched. In vertical sections through young galls 12–25 epidermal cells were counted, whereas in more mature septa the number ranged between 30 and 45. The mesophyll achieves temporary accommodation to the lateral strain through the elasticity of its cells. Possibly the endurance of the epidermal layers signifies that in this case adjustment to continued strain is more efficiently coped with by cell division than by cell plasticity. It seems certain that growth tension is a factor in modifying the septum, but the rôle played by aphid suction appears dubious.

Summary

1. Two conical "up-walled" chambers, produced by one aphid, develop concurrently on opposite sides of the leaflet, and a partition consisting of modified leaf tissue is left as a septum between the compartments.

2. Within the septum the tissues approximate a homogeneous condition, and remain as relatively inactive meristem, in which some hypertrophy is manifest, but in which only a very little hyperplasia occurs.

3. The septum is an evanescent structure, and in its deterioration the mesophyll cells attenuate horizontally and disappear, bringing the two epidermal layers into a temporary union. Eventually the epidermal layers break and the two gall cavities become one.

4. Growth tension appears to be the main factor in transforming the septum.

5. In the region bordering on the septum, the meristem, dedifferentiated from the original layers, expresses itself in prolific activity. Reversion to the primitive meristem condition is rapid, and is

accompanied by extensive hyperplasia. Very pronounced hypertrophy ensues in most of the cells.

6. In point of diversity this gall displays limited tissue differentiation. A nutritive zone, an extensive parenchymatous region containing vascular tissue, and an epidermal layer covering certain parts of the gall's surface are the chief tissues.

7. Setal punctures are numerous within one gall. Sheaths are often associated with the punctures.

8. Puncture channels were found frequently in tissues of active proliferation, as well as in regions of retarded growth.

9. Greatly enlarged starch grains develop in some cells of the parenchyma.

The writer is indebted to Dr. B. W. WELLS, of the Botany Department of North Carolina State College, for information and much considerate assistance in this study.

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DESCRIPTION OF PLATES XXII–XXV

PLATE XXII

FIG. 1.—Upper cone of very young gall.

FIG. 2.—Lower cone of very young gall.

FIG. 3.—Vertical section of gall shown in figs. 1 and 2, showing septum between two developing chambers.

FIG. 4.—Gall which is nearly mature.

FIG. 5.—Vertical section of gall shown in fig. 4; septum rim present in center.

FIG. 6.—Vertical section through normal young leaflet, showing characteristic tissue arrangement in five rows.

FIG. 7.—Vertical section through more mature leaflet, drawn within walls of greatly hypertrophied cell; both leaf tissue and hypertrophied cell drawn to same scale of magnification.

FIG. 8.—Enlarged starch grains in cells of gall parenchyma.

FIG. 9.—Cells of *Phylloxera caryaecaulis*, showing many enlarged starch grains.

PLATE XXIII

FIG. 10.—Extremely large starch grains in different region of same gall used in fig. 8.

FIG. 11.—Very young gall in vertical section, in which was abnormal horizontal division in upper epidermis of septum; in this stage mesophyll cells of septum are seen to be rather cubical.

FIG. 12.—Septum of fairly young gall, showing horizontal elongation of cells.

FIG. 12*a*.—Outline of vertical section of fairly young gall; shaded portion represented in detail in fig. 12.

FIG. 13.—Vertical section of normal young gall.

FIG. 14.—Unusual type of gall, in which aphid is present in each chamber.

FIG. 15.—Immature stem mother.

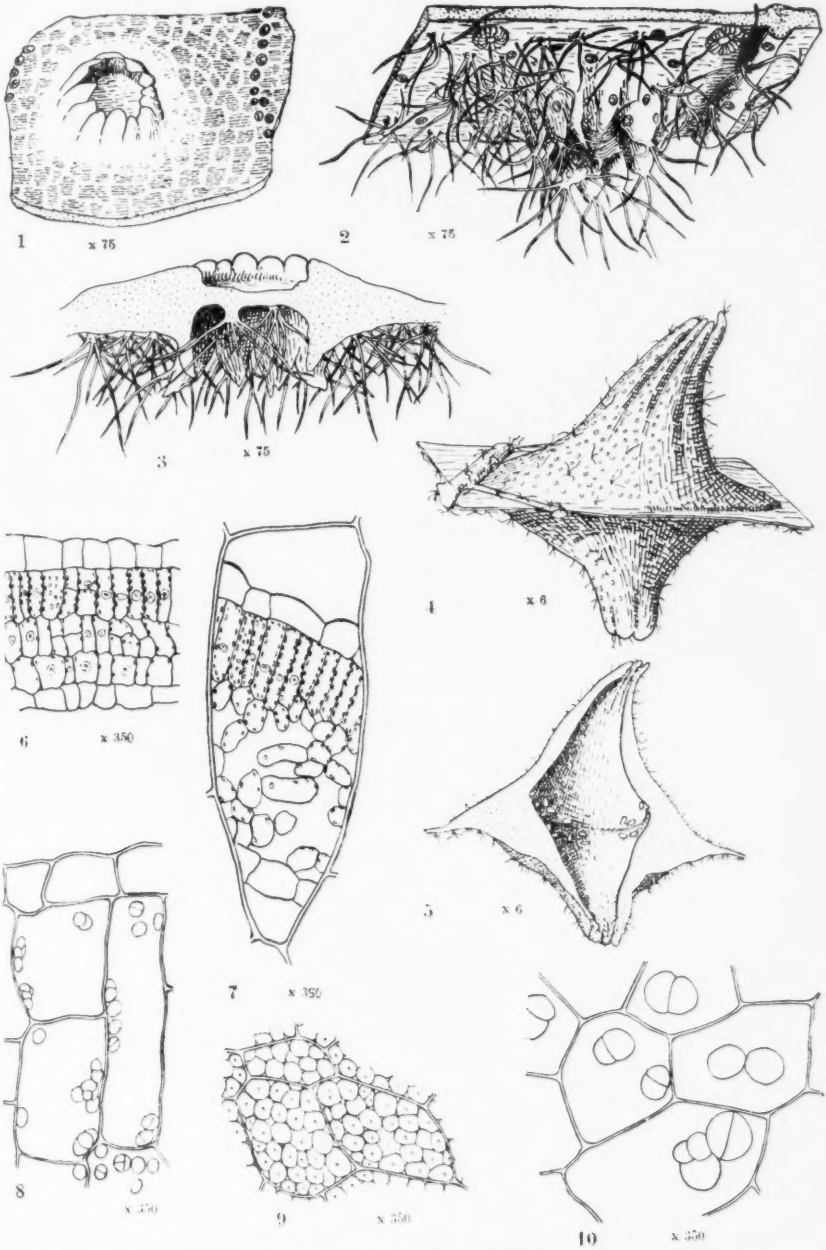
PLATE XXIV

FIG. 16.—Mesophyll cells of septum showing collapse, bringing the two epidermal layers into contact.

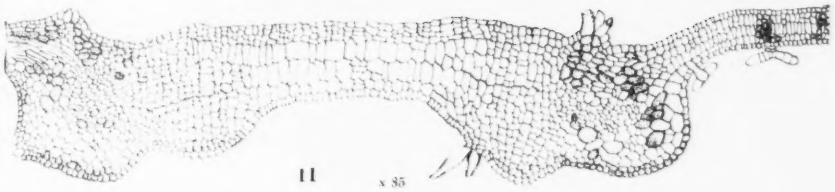
PLATE XXV

FIG. 17.—Vertical section through part of cavity and walls of mature gall; nutritive zone and remnant of septum indicated.

FIG. 17*a*.—Shaded portion of this gall drawn in fig. 17; upper nipple of gall does not show because section is not perfectly median.



MARTIN on PHYLLOXERA



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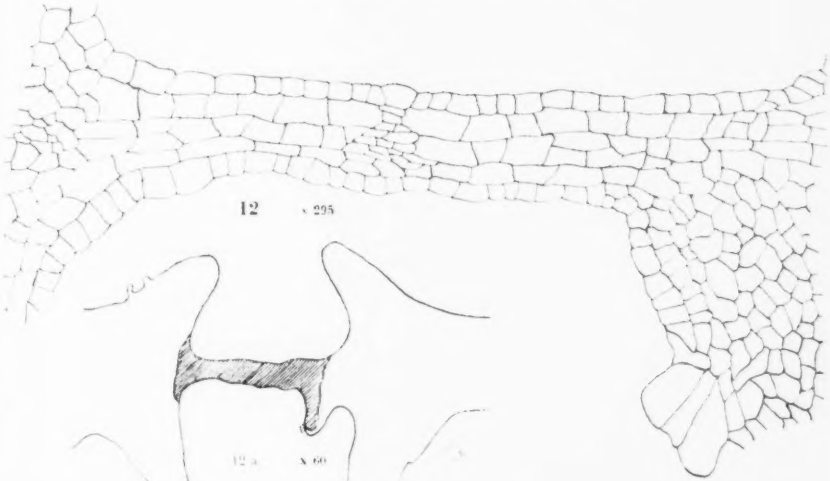

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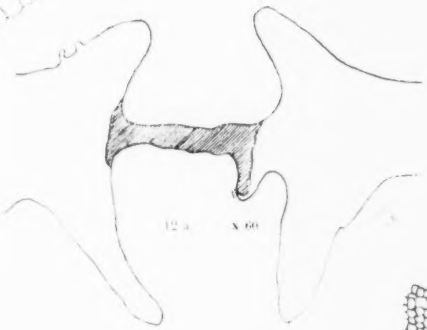

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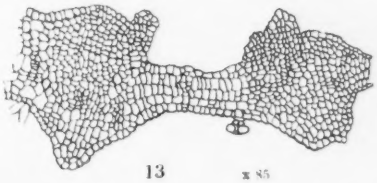

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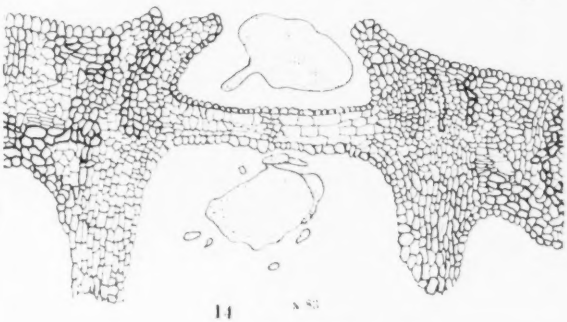

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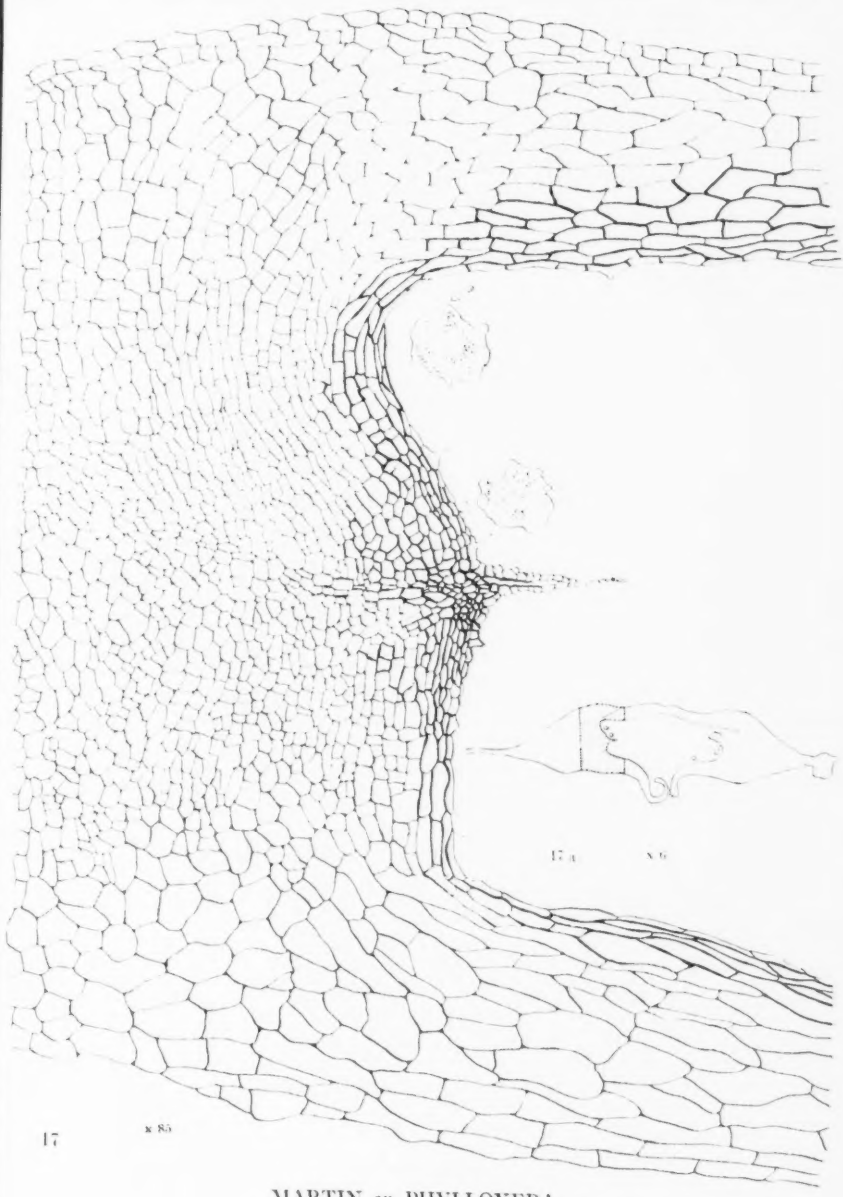
MARTIN on PHYLLOXERA

MARTIN on PHYLLOXERA



MARTIN on PHYLLOXERA

GROWTH STUDIES IN FOREST TREES[1]

III. EXPERIMENTS WITH THE DENDROGRAPH ON *FRAXINUS AMERICANA*

J. ELTON LODEWICK

(WITH TWO FIGURES)

Two previous papers in this series have been published by BROWN (**2, 3**). Upon his suggestion the writer is continuing investigations along similar lines, and has adopted the same title for further contributions to the knowledge of growth in trees.

Very little has been published as to the season of cambial activity in the broad leaved deciduous trees of the northeast, although the experiments of MACDOUGAL and his co-workers in the west and southwest have yielded some interesting results, and show promise of many more. In order to render these most useful, however, they should be checked by histological studies carried on simultaneously, but to date no such work has been published, and interpretations of the growth curves obtained have been based wholly on dendrograph records, unsupported by microscopical data. During the season of 1923 such studies were undertaken by the writer, to determine the accuracy of the growth curves obtained with the dendrograph, and to further our knowledge of the value of such records in computing xylem increment. In addition, a growth intensity curve showing a few points of interest has been plotted.

The dendrograph, one of the latest models, was procured from D. T. MACDOUGAL by the New York State College of Forestry. The structure and operation of this instrument have been fully described by the maker (**9**), but a brief summary may be helpful. It may be considered as composed of three portions: a basal belt of blocks, a floating frame, and a recording device (fig. 1). The first is a series of wooden blocks so hinged together at the ends that the whole may be placed around a tree and drawn tight by means of two

[1] Contribution from the Department of Wood Technology, New York State College of Forestry, Syracuse, N.Y.

thumbscrews. This belt acts as a support, and serves to hold the recording instruments in the same relative position to the tree under all conditions. The second portion is a polygonal frame of narrow invar bars, which are slotted at the ends and clamped solidly together by special bolts. This may be composed of any even number of bars above four, depending upon the diameter of the object measured; six were necessary to encircle the tree used in the present in-

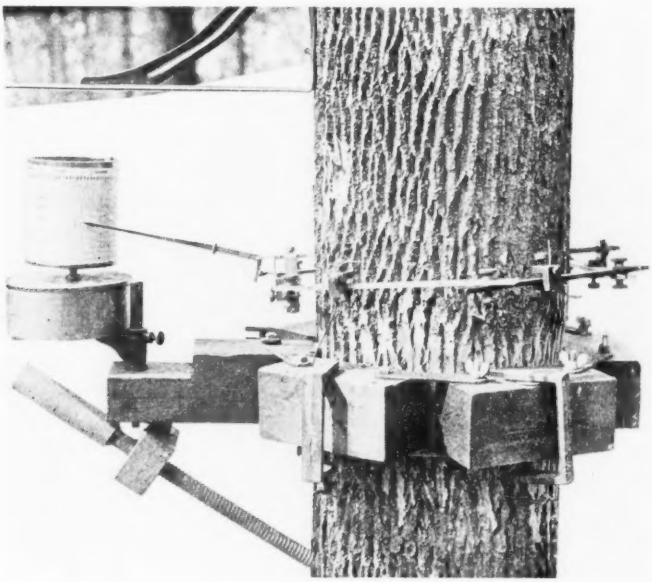


FIG. 1.—The dendrograph

vestigation. This floating frame, so-called because it is not directly in contact with the tree at any point, is supported about three inches above the basal belt on four slender wires. At the center of one bar is an adjustable contact screw, blunt at the apex, which is in contact with the tree, and fastened to the bar at the base. Diametrically opposite to this the frame bears a movable quartz rod, in contact with the tree at one end and operating against the short arm of a pen-bearing lever at the other. The rod is so arranged that it can

be brought to bear at any of three points along the short lever arm, thus giving three possible magnifications of the diameter fluctuations of the tree. The third and recording portion of the instrument consists of an eight-day clock operating a cylinder covered with a ruled record paper, and attached to a large block in the supporting belt.

The tree selected was a forest-grown specimen of *Fraxinus americana* L., growing in a sandy loam deficient in humus, and on an east slope at the College Experiment Station about three miles south of Syracuse, New York. This individual was approximately fifty years of age, thirteen inches in diameter, about forty feet tall, and free of branches for eighteen feet. The crown was well lighted from above, but one-sided because of shading on the north and east. The dendrograph was attached at breast height to measure the north-south axis, and adjusted to magnify diameter changes 12.5 times.

Probable errors in dendrograph

Four apparent opportunities for error are present in the dendrograph because of its structure and method of operation. The first, the variation resulting from the coefficient of expansion of the materials used, has been shown by MacDougal to be negligible. Computations of the possible variation under normal daily temperature ranges show it to be too slight to be recorded, even under the highest magnifications obtainable.

All growth taking place under the movable quartz contact is registered directly, but the diameter increase on the opposite side of the trunk can be recorded only by a movement of the whole floating frame toward that side of the tree. To move the frame thus, the supporting wires must be bent, hence some pressure is exerted under the stationary contact screw in order to overcome this inertia. It was thought possible that some atrophy might occur under the contact screw as a result. On October 8 the dendrograph was removed and a series of cuttings made at this point, controls being taken at distances of one inch on all four sides. Careful measurements of the ring width with the microscope failed to show a narrowing of the xylem increment because of applied pressure, eliminating this factor as a probable source of error.

During the season the basal belt of blocks was not loosened; hence some pressure and consequent atrophy might have occurred under them. If the constriction were great enough to retard food transportation, the ring should be narrower under and below the blocks and wider above, and the increased growth might be transmitted far enough to be recorded by the contacts. Three series of cuttings were made when the instrument was removed in October for these tests. Each series began below a basal block and ended about two inches above the floating frame, with cuttings taken at intervals of about an inch. In none of the three series, taken under different blocks, was a change noticeable in the ring width; hence the dendrograph apparently does not interfere with normal food transportation and causes no modification of the ring width.

Because of the heavy furrowed bark of *Fraxinus americana*, it was necessary to cut away small areas of the periderm to provide smooth bearing surfaces for the contact rods, as well as to reduce to a minimum any hygroscopic changes in this layer caused by atmospheric moisture. The white living inner bark of the tree was approximately 5 mm. thick, and the contact points were placed directly on the outer portion of this layer. Drying of the exposed tissues was to be expected and did occur during the first week. Later a new layer of periderm formed, and cuttings from these areas in the autumn indicated that drying and consequent checking had occurred to a depth of about 2 mm., but was never deep enough to interfere with cambial activity. This justifies the assumption of MACDOUGAL that excessive drying would not follow exposure of the moist inner phloem. The newly formed periderm was not thick enough to affect materially the growth curve.

Progress of growth

The dendrograph was set up on April 11, but the record was of no value until a week later because of adjustments of the instrument necessitated by the drying of the exposed living tissues. After April 18 compensating variations were evident, and the logical supposition is that these were in progress previously.

The daily variations observed by other workers in all of the species investigated were present. On clear days, especially those which

were warm and windy, the curve showed a decided drop between 8 A.M. and noon, which continued until about 6 P.M., then rose slowly to a maximum after midnight, thereafter remaining constant until the drop of the following noon. MACDOUGAL has assumed this noon depression to be an indication of decreased turgor, and to be due to the rapid transpiration of midday. A water depletion in the stem develops, since the roots absorb water at a uniform rate and fail to maintain the usual water balance. If this assumption is correct, the noon depression should be at a maximum when the tree is in full leaf, and it follows that the greater the capacity of the air to absorb water, the greater should be the depression, all other factors being equal. The average drop of midday computed from the data for the season of 1923, from April 18 until the day that the buds broke open (May 6) was 0.54 mm. Between the latter date and September 22, the time of complete defoliation, the average depression was 1.64 mm., or roughly three times as great. From September 22 until the end of November the daily average was only 0.19 mm. No reason for the discrepancy between the depression before the growing season and that after defoliation can be advanced, other than that an appreciable evaporation takes place from the swelling buds. The depression during the period of leaf unfolding tends to average slightly higher than that occurring when the leaves are completely expanded. Whether or not this is dependent upon the structural development of the leaves and twigs must be reserved for future investigations.

The evaporating power of the air is dependent mainly upon temperature, humidity, air movements, and sunshine. An examination of these data for the season shows some correlation between the amount of noon depression and the power of the air to absorb water, but no definite figures can be given because of the inability to assign a constant to each factor. Further investigations covering these factors are planned for the ensuing season.

With only one growth intensity curve covering a single season it is inadvisable to draw conclusions as to the effect of external conditions upon the growth rate. Attempts were made to correlate daily changes with meteorological data, but without results. Several investigators, especially FRIEDRICH (**5**) and KORSTIAN (**8**), have in-

dicated that a decided drop in temperature is followed by a decrease in the growth rate. This must act as a shock, because in the cases observed the temperature did not reach a point sufficiently low to inhibit growth. On June 5 and June 27 respectively, a decrease in temperature great enough to cause a decided decrease in the growth rate was observed. On the two succeeding days in each case the growth intensity again increased, but the temperature continued to fall. Before and after the period of diameter enlargement the compensating variations appeared to be closely related to precipitation, as shown by the growth-intensity curve (fig. 2). An appreciable precipitation is followed shortly by an increase in diameter, which is lost following the rainy period. This is not to be considered as growth, since no permanent increase in size results. On the other hand, such a phenomenon might be caused by hydration of the bark layers during times of high humidity, but the irrigation experiments of MACDOUGAL eliminate this factor, yet give quick increases in diameter.

Histological studies

Histological studies, aiming to correlate the dendrograph record with the progress of growth within the tree, are probably the most needed investigations at the present time. Cuttings were made at intervals of about two weeks in a manner similar to that used by BROWN (**2**). Small pieces showing phloem, cambium, and xylem were removed from near the height of the floating frame and close to the bearing points. Two objections to this method are possible, namely, the effect of removal of pieces on reduction of the internal pressure, and the effect of callus formation on the size of the annual ring in subsequent cuttings. Any decrease in tension through cutting should be evident in the dendrograph record, but this was not observed. The second objection was circumvented by making all the cuttings outside the range of callus formation. In addition, no incisions were made sufficiently close to the bearing points that the added growth resulting from callus formation would be effective in these areas.

The pieces removed from the tree could not be sectioned without imbedding because of the thin walled cambial layer, hence the tissues were killed and fixed in chromo-acetic fluid, softened in hydrofluoric

acid, imbedded in celloidin, and studied with the aid of both temporary and permanent stains.

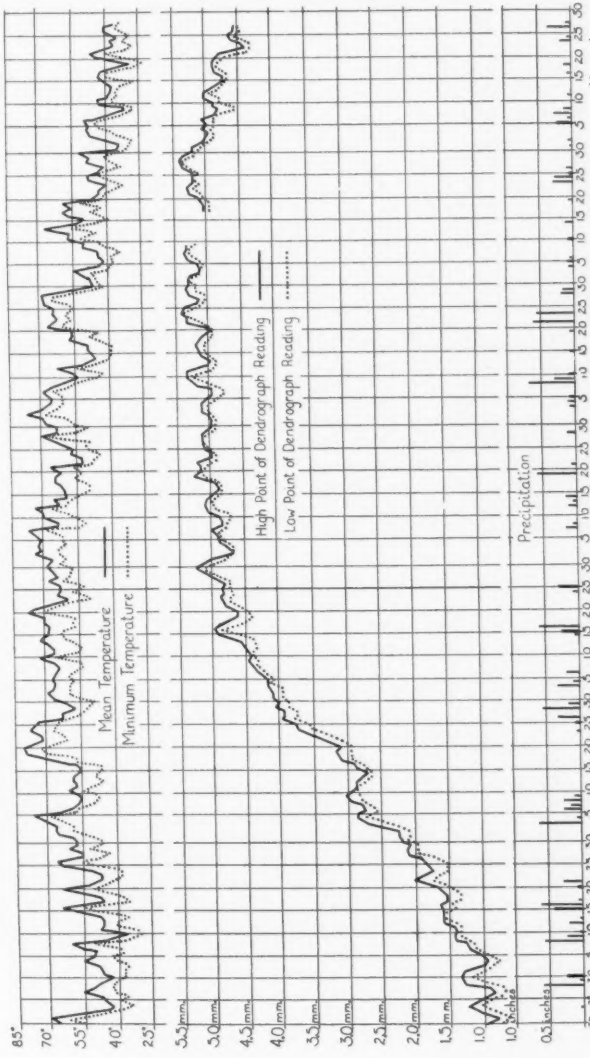


FIG. 2.—Growth intensity curve during 1923, as plotted from dendrograph records

The buds on the ash were swelling noticeably on April 20, and had broken open on May 6; the leaflets were unfolding on May 15, and were one-fourth to one-third full size on May 30. The tree may be considered as in full leaf in mid-June. The first cutting, taken May 9, showed that the first row of springwood vessels had been formed, but the vessel walls were still thin and unlignified. On May 30 the second row of vessels was developing, but as yet only the primary wall was visible. The spring wood zone was complete on June 13, so far as number of cells was concerned, and nearly half of this was still unlignified. On July 11 the last row of summer wood ducts had just arisen from the cambium; while a subsequent cutting on August 9 showed a ring complete so far as cell number was concerned, but with the major part of the summer wood fibers still unlignified. Later cuttings showed no change in the annual ring, except for wall thickening and accompanying lignification.

The beginning of phloem formation appears to vary in different species of trees. STRASBURGER (12) indicated that in general xylem elements formed some time before the phloem elements in the spring, and that the phloem continued to develop until the end of the vegetative season. PFEFFER (11) reached the same conclusions. BROWN (3) concluded that in *Pinus Strobus* phloem and xylem formation begin simultaneously, and that phloem continues to be formed until the end of the growth period.

In *Fraxinus americana* the end of seasonal activity is marked in the phloem by two rows of radially compressed cells. The measurements of phloem widths at various times during the growing season are recorded in table I. It is evident that the first phloem elements of the season are formed several weeks after the first xylem cells are differentiated. It will be shown subsequently that it is reasonable to conclude that phloem development continues at least until defoliation in the autumn.

The first point at which the curve of growth intensity portrayed an increase above the higher points of previous compensating variations was on May 8, and cuttings taken on the following day indicated an increase in xylem, as previously stated. It does not seem probable that all this growth could occur in one day, and it is logical to conclude that cambial activity started at breast height in the bole

some time between April 29, the time of the last high point dependent on swelling of the tissues, and the date on which growth was actually recorded. If this is true, cell division must have taken place when the tissues were not fully distended. As has been stated, the buds were breaking about May 6, hence cambial activity in the base of the trunk began simultaneously with this phenomenon. After growth had once started, it continued at a fairly even rate until June 9; then a rest period of approximately ten days was evident, accompanied by shrinkage in diameter. It is to be noted that this was also a period of decreased temperatures and no precipitation. If either of these factors is to be considered as causing the rest

TABLE I

COMPARISON OF DIAMETER INCREASES REGISTERED BY DENDROGRAPH AND MEASUREMENTS OBTAINED FROM CUTTINGS

Date	Width of xylem (mm.)	Width of phloem (mm.)	Width of cambium (mm.)	Total diameter increase (mm.)	Total diameter increase according to record (mm.)	Percentage growth shown by cuttings as recorded
May 9.....	0.351	0.078	0.000	0.854	0.100	11.7
May 30.....	0.577	0.078	0.202	1.714	0.850	49.6
June 13.....	0.858	0.062	0.310	2.460	1.700	65.0
July 11.....	1.840	0.062	0.429	4.062	3.150	67.5
August 10...	2.292	0.062	0.449	5.606	3.900	69.5
October 8....	2.341	0.062	0.468	5.742	4.050	70.7

period, it would of necessity be the temperature, since the resumption of cambial activity was coincident with the approach of higher temperatures, but preceded precipitation by nearly a week. Moreover, several days of precipitation occurred just before the cessation of cambial activity, so that the soil undoubtedly contained plenty of water for growth phenomena. FRIEDRICH (**5**) and JOST (**7**) have noted a similar resting period in hardwoods and conifers, but their methods are open to criticism. MISCHKE (**30**) observed the same phenomenon in *Pinus silvestris* and *Picea excelsa* during the latter part of June, and attributed it to the low precipitation of preceding months. BROWN (**3**) found a comparable cessation of cambial division in *Pinus Strobus* at the same time of year, and concluded that the first period of cambial activity is at the expense of stored food reserves, while the second results from food manufactured in the

new leaves. Such an explanation seems more plausible in white ash, since the tree was not in full leaf until mid-June, which date just preceded the beginning of the second growth period. It is worthy of note that the rest period occurred between the formation of spring wood and summer wood elements.

Growth intensity was high during the first part of the second growing period, but gradually decreased toward its close. A period of rest is evident between July 16 and 27, for which no reason can be assigned. Few xylem elements were formed later than August 1, after which date only equalizing variations were present. On September 19, however, a new maximum was established, 0.1 mm. higher than that of July 30, and this was further increased on September 24 by 0.05 mm. It should not be inferred, however, that these new maxima were the result of xylem formation. Previously mentioned investigators, especially BROWN (3), have noted the continued formation of phloem elements in various species until inhibited by the advent of low temperatures incident to autumn and early winter. The phloem in *Fraxinus americana* is so irregular that no definite counts of phloem elements could be made to determine whether or not such an explanation would account for the new maxima in this species Examination of the cuttings taken during this period indicated that such a conclusion was plausible. The break in the curve in October resulted from readjusting the instrument upon the tree, following its removal to obtain the test cuttings already described.

After the end of October a more or less steady decrease in diameter was registered. This may have been caused in part by the release of pressure, and in part by drying occasioned by the large number of cuttings which had just been made. On the other hand, MACDOUGAL has observed such a contraction in the trunk in all species examined by him; hence a decrease may normally be expected at this time, although the amount is probably influenced by the factors mentioned.

Interpretation of growth records in terms of xylem increment

The ring width on different dates was determined from the cuttings made near the stationary contact screw on the south side of the tree. Xylem, cambium, and phloem widths were computed, and doubled to give the total diameter increase, with the results given

in table I. From these data there is no apparent agreement between the diameter increment as recorded and as measured. This may be explained in part by swelling consequent to the preparation of permanent mounts. FRENCH (4), in attempting to determine the specific gravity of wood by computing the actual volume of cell walls on cross-sections, found that his results were consistently high. He concluded that swelling to the extent of twenty-five per cent had taken place during the processes involved in the preparation of his slides. BROWN (1) determined that in wood of *Tecoma* treated with hydrofluoric acid, an average increase of 29 per cent resulted in the radial direction, and was accompanied by a longitudinal decrease of over 20 per cent, and a tangential increase of over 40 per cent. If similar changes took place in *Fraxinus americana*, the measurements of column 4 in the table should be reduced about 30 per cent, and would then correspond more closely with those of the dendrograph record.

The earlier cuttings of the year show a greater discrepancy, the dendrograph recording but 12 to 15 per cent of the growth as computed from permanent mounts. As stated, a portion of this might be attributed to a swelling of the tissues resulting from desilicification. The remainder is possibly due to an irregular awakening of the cambium. In general, cambial activity on the south side of trees precedes that on the north, because of better insolation; but growth, once actuated, progresses more rapidly on the north side. Near the end of the growing season the ring width is comparable at the two points. All measurements in this investigation were made on the south side, and it is possible that growth was more tardy on the north side; hence the discrepancy obtained by doubling the measurements. Further investigations are necessary, and these explanations of differences between the dendrograph record and actual measurements are advanced only as possibilities, and not as conclusions drawn from actual data at hand.

Summary

1. Growth studies were made during the season of 1923 on *Fraxinus americana* L. by means of a dendrograph. Correlated histological investigations were made on the same tree.

2. No distortion of ring width is caused by the contact points or basal blocks of the dendrograph.

3. The desiccation of layers of phloem exposed in setting the instrument does not interfere with cambial activity.

4. The noon depression in diameter is greatest when the tree is in full leaf, supporting the supposition that this is brought about by the water relations of the tree.

5. A decided drop in temperature is usually followed by a decrease in or a cessation of growth.

6. The compensating variations before and after the growing period are closely correlated with precipitation.

7. The first xylem elements at the base of the trunk were formed simultaneously with the breaking of the leaf buds. Xylem formation ceased in this region about August 1.

8. Phloem formation began about three weeks after the first wood elements were formed, and continued at least until the defoliation of the tree on September 22.

9. A rest period of ten days occurred in early June which could not be correlated with external factors.

10. The dendrograph registers less growth than is shown by cuttings, but no definite explanation can be given for this phenomenon.

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FORMS OF NITROGEN IN PURE CULTURES OF ALGAE[1]

E. B. Fred and W. H. Peterson

The growth of algae in pure culture has been the object of many studies (**2**,**7**). In general this work has dealt with the question of free nitrogen fixation, without taking into account the composition of the algae when grown in pure culture. In nature a profuse growth of the algae is not uncommon, and yet the algae mass which develops in this way usually is associated with various forms of life. In this report are presented the results of a study of methods for growing large quantities of algae in pure culture, and also some of the forms of nitrogen found in algae.

Technique

The method for the isolation of green algae is similar to that given in detail by Schramm (**7**). His culture technique has been followed in this report with certain modifications. A large volume of water from the surface of Lake Mendota (100 liters) was run through a Sharples supercentrifuge at about 40,000 r.p.m. This deposit from the sides of the bowl was diluted and agar plates of the various dilutions poured. The results from the picking of single cells by means of the Barber (**1**) or Chambers (**3**) apparatus agree with those of Schramm; it is a slow method without any decided advantages. After several trials of various media, the following were used (**4**).

1. Chodat and Grintzesco medium

$Ca(NO_3)_2$	1.0 gm.
K_2HPO_4	0.25 gm.
$MgSO_4$	0.25 gm.
KCl	0.10 gm.
$FeSO_4$	Trace
Water, distilled	1000 cc.
Agar, washed	10.0 gm.
Reaction	P_H, 5.3–5.5

[1] Published with the permission of the Director of the Agricultural Experiment Station, Madison, Wisconsin.

2. MODIFIED MEDIUM

$Ca(NO_3)_2$	1.0 gm.
KH_2PO_4	0.2 gm.
$MgSO_4$	0.2 gm.
$CaCl_2$	0.1 gm.
$FeSO_4$	Trace
Water, distilled	1000 cc.
Agar, washed	10.0 gm.
Reaction	P_H, 7.3

A large number of plates of high dilutions were poured. The plates were inverted, placed under a belljar in a north window, and allowed to incubate for several weeks. From well isolated colonies many cultures were picked and streaked on the surface of agar slopes. From the slopes which showed a profuse growth of algae, transfers were made into glucose broth and glucose yeast water. When subjected to this test the contaminated cultures were easily detected. To secure maximum growth of algae in a short time, additions of glucose or sucrose to the CHODAT and GRINTZESCO medium were used. These carbohydrates proved very beneficial, and it was found that after 60 days incubation the sugars were almost entirely assimilated. Large amounts of the algae growth were obtained by the use of a number of tall 16 oz. flat-shaped bottles, to which 75 cc. each of liquid medium was added. After inoculation these cultures were kept in the greenhouse, flat side down. The necks of the bottles were plugged with cotton and covered with paper caps.

Experimental

GROWTH OF ALGAE IN LARGE QUANTITIES.—In connection with some studies on vitamin synthesis by micro-organisms, as well as for nitrogen analysis, a large quantity of algae was grown. A report of the vitamin studies will be given at a later date. A series of flasks and bottles with shallow layers of liquid culture media with and without glucose were inoculated with pure cultures of *Chlorella vulgaris* and *Scenedesmus quadricauda*. The plan of the experiment and the growth of the algae are shown in table I. Similar experiments were carried out with other strains of algae, but due to contamination with bacteria these were discarded. The results shown in table I

were obtained with cultures free from bacteria. At the end of the incubation period these cultures were tested for presence of bacteria. It will be seen that in the series to which glucose was added the development of the algae was far in excess of the cultures without sugar. In the absence of glucose, *Scenedesmus* grows much better than *Chlorella*. In the last column it will be seen that the algae are unusually high in mineral matter. This large percentage of ash was due in part to insoluble salts of the culture medium thrown out by the supercentrifuge. The total dry weight of the harvest was great

TABLE I

GROWTH OF *Chlorella* AND *Scenedesmus* IN LIQUID MEDIA

No.	Name	Medium	Number of cultures	Age in days	Dry weight (gm.)	Percentage moisture	Percentage ash
1.....	Chlorella vulgaris	Chodat and Grintzesco	150 flasks, 100 cc. in each	128	1.03	.....	.....
2.....	Scenedesmus quadricauda	Chodat and Grintzesco	200 bottles, 75 cc. in each	88	7.85	84.0	38.0
3.....	Chlorella vulgaris	Modified plus 1% glucose	190 bottles, 75 cc. in each	20	11.3	83.0	20.8

enough to justify further work along this line; accordingly several experiments were carried out and the algae obtained saved for chemical analysis.

FORMS OF NITROGEN IN CHLORELLA.—In dissecting the forms of nitrogen present in *Chlorella* sp. grown in the absence of bacteria, the algae cells were first thrown out of the liquid medium by centrifuging, and this material scraped from the side of the bowl and dried at 65° C. When dry the material was ground to a fine powder in a mortar, and the nitrogenous constituents extracted by macerating with water, as described by TOTTINGHAM, SCHULZ, and LEPKOWSKY (**9**). The soluble nitrogen was separated into two portions, protein and non-protein nitrogen, by means of FOLIN and WU's (**5**) tungstic acid precipitation. This reagent was found by HILLER and VAN SLYKE (**6**) to precipitate the proteins and most of the intermediate products. Trichloracetic acid was used for comparison, as the latter reagent was reported to precipitate the proteins and

leave the intermediate products in solution. Free and combined amino nitrogen were determined by VAN SLYKE'S method before and after hydrolysis; the nitrates according to the method described by STROWD (**8**). The data are given in table II, and show that about 25 per cent of the total nitrogen is water soluble. About one-third of the water soluble nitrogen consists of nitrogen in the form of proteins and intermediate products, one-fourth is made up of amino nitrogen, and one-fourth is contained in peptide linkages.

TABLE II

FORMS OF NITROGEN FOUND IN PURE CULTURES OF *Chlorella vulgaris* DRIED AT 65° C., CALCULATED FOR 100 GM. ASH FREE MATERIAL*

FORMS OF NITROGEN	WEIGHT OF NITROGEN		PERCENTAGE DISTRIBUTION	
	Trichloracetic pptn. (gm.)	Tungstic acid pptn. (gm.)	Trichloracetic pptn.	Tungstic acid pptn.
Total nitrogen	7.409	7.409	.....	.....
Soluble nitrogen	1.457	1.457	23.9	23.9
1. Soluble nitrogen ppt. by reagent	0.351	0.531	5.3	8.7
2. Forms of soluble nitrogen in filtrate				
a. Free amino nitrogen	0.329	0.357	5.4	6.1
b. Combined amino nitrogen	0.541	0.364	8.8	6.2
c. Nitrate nitrogen	0.069	0.069	1.4	1.4
d. Rest nitrogen	0.167	0.115	2.9	1.3

* Ash in original material 17.6 per cent.

Conclusions

1. Pure cultures of *Chlorella vulgaris* and *Scenedesmus quadricauda* were isolated from the water of Lake Mendota and grown in large quantities.

2. The nitrogen in the dried algae is largely insoluble and amounts to 7.4 per cent on the ash-free basis. About 25 per cent can be extracted with water, and is made up chiefly of protein, amino, and peptide nitrogen.

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CORRELATION AND CYCLIC GROWTH IN PLANTS

A. E. MURNEEK

Guided by the concept that fundamentally growth is a chemical process, there has come into existence a school of biologists, BLACKMAN (**2**), LOEB (**6**), ROBERTSON (**12**), and others, who have attempted to demonstrate that the rate of growth of an organism, when expressed as increments in volume, height, dry weight, etc., gives a curve that closely resembles and in some instances is almost identical with that of a monomolecular autocatalytic reaction. Hence growth has been considered as a catalytic process, and the organism as the end product resulting from the action of an autocatalyist on a subtrate.

While these deductions have been based largely on data obtained from measurements of growth of animals, the theory has been expanded by several workers (REED and HOLLAND **11**, ROBERTSON **12**) so as to include plants as well. Thus the former have shown that in the case of *Helianthus*, when growth is expressed by increase of height of the stem, the measured values agree remarkably well with those calculated, when the usual formula of a monomolecular autocatalytic reaction is employed. In a recent discussion of the mechanism of growth, ROBERTSON marshalls this and similar evidence as proof of the fundamental nature of growth of all organisms.

As to the exact mechanism or the character of the essential catalyst in operation (for the type of the curve suggests such to be operative) various theories of hypothetical substances have been advanced, either catalytic, or inhibitory, or both, as responsible for the "master reaction" ("formative substances," "catalyzers," "inhibitors" of LOEB; "inhibitors" of REED; "autocatalyst" of ROBERTSON; "bios" of WILDIERS). From the present status of the question it is evident that the whole matter is still in a problematical if not hypothetical stage.

Additional information on the nature of this rather general subject has been accumulated by the writer during the past two

years, as a result of extensive investigations on growth and yield in the tomato (*Lycopersicum esculentum*). It shows that growth in at least this plant, and probably in many others, may be more readily explained on the basis of correlation. Complete evidence has been obtained that correlation is one of the most important if not the main factor concerned with the rate of growth in plants. While at present only the comparatively late stages in the development of the tomato have been taken into consideration, that is, those commonly expressed by the "autostatic" phase of the curve, striking proof has been obtained that this phase of the autocatalytic curve is produced by the effects of correlation of the fruit on the rate of growth of the main stem. It has been found that: (1) Plants grown without fruit have no such phase during the period when it normally occurs; the curve is absolutely straight. (2) The presence of fruit introduces this phase at once, the growth of the plant then running parallel with that of any normal fruiting plant. (3) The "autostatic" phase can be introduced at will by deliberately putting the plant either in a vegetative condition (flowers or fruit removed) or in a reproductive condition (normal check plants). In respect to growth, such plants during the vegetative period show a striking parallelism with strictly vegetative plants (flowers removed), and during the reproductive period with strictly reproductive plants (flowers and fruit present). (4) Normally growing plants, provided there are enough nutrients (nitrogenous) left either in the plant or the soil, will upon ripening of fruit at once commence to grow, if no other immature fruits are present, or if present are not in large numbers and not too close to the growing points. This has been found to be true in the tomato under various conditions of nutrition. It is exhibited in carbohydrate high plants particularly well; terminal growth is not only inhibited but often totally checked or destroyed by the developing fruit. Moreover, it has not been possible to disarrange the mechanism by submitting the plants to even as short an exposure to light each day as two and one-half hours. Then, too, it has been found that the effects of correlation may be more or less localized, one-half of a two stemmed plant responding to treatment, while the other half is serving as a control. Thus the evidence is clear (1) that vegetative growth in the tomato diminishes at the exact time and in exact

proportion to the amount of flowers formed and fruit set, and (2) that the rate of vegetative growth appears to be controlled by the developing fruit. The latter fact is of particular importance. It is a conception contrary to our current point of view. In the consistent gathering of evidence on the effects of vegetative growth on reproduction, we seemingly have neglected to analyze a reverse phenomenon, which is just as striking. Hence this may be a timely emphasis on the value of experimental treatment of the reproductive parts of the plant, particularly perennials, and the observation of the resultant response in both vegetation and reproduction.

That other plants behave similarly to the tomato may be gleaned from the careful analytical records on growth and yield of cotton by BALLS and HALTON (1) and by MASON (7). The extensive statistical investigations by the former show that the rate of vegetative growth in the cotton plant diminishes at the exact time and in exact proportion to the amount of flowers formed and fruit set. These results the authors unsuccessfully attempt to explain as being due to environmental factors, whereas apparently quite similar observations are correctly interpreted on the basis of correlation by MASON. In fact, MASON'S experimental data and a part of his conclusions, although secured independently and based upon a different plant, are strikingly similar to those obtained by the writer. This agreement is certainly very suggestive of the importance and fundamental nature of correlation in growth of plants.

The "autokinetic" phase of the growth curve may probably find its counterpart of explanation in the influence of correlation of roots on the stem (BRENCHLEY 3, PEARSALL 9).

This evidence, although furnished largely by the tomato and to some extent by the cotton plant, tends to show that growth, as measured by increase in length of stem, is determined during the early stages probably by the development of roots and later certainly by the flowers and fruits. Hence a curve is obtained suggestive of that of an autocatalytic reaction. Moreover, such conception agrees with our present knowledge of the periodic or cyclic growth of the various parts of the body or the different organs of animals. Evidently in this respect also there is after all no fundamental difference between plants and animals. It is interesting to note that from a

mathematical interpretation of the rate of growth of corn, PEARL and SURFACE (8) conclude that there are distinct cycles of growth of the various parts of this plant, which probably are of the nature of correlations. Recently REED (10) also has pointed out that lateral growth of apricot branches is distinctly cyclic.

What, however, is the mechanism through which growth correlations find expression in the plant? Have we here evidence of localized nutrition giving rise to a metabolic gradient, such perhaps as suggested by CHILD (4)? Is it possible that sexual reproduction or gametic union may create a really extraordinary rejuvenescence in organs closely allied to the developing embryo, which results in reduction or inhibition of growth in other parts of the organism? But how is the developing embryo or fruit able to monopolize practically all of the incoming or synthesized material? Do plants have a controlling glandular organization or a system of internal secretion similar to that existing in animals? Apparently the basic part of the problem is either one of nutrition or of special secretion at certain stages of the development of the plant, leading to localized acceleration in metabolism and the rapid development of particular organs. In the absence of any proof of an internal secretory system in plants, the writer is inclined to believe that the phenomenon may be the outcome of different stages of localized nutrition, and that the carbohydrate-nitrogen relations, as proposed by KRAUS and KRAYBILL (5), may be used as a key in interpreting these rather striking conditions of growth in plants. Even if the response perchance is due to special secretion, a careful determination of some of the physiological and chemical alterations in the participating organs ought to reveal a closer picture of the true situation. With this in mind, a series of chemical analyses of various parts of the tomato plant is in progress at present.

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DISPERSION OF LIPOIDS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 335

DEAN A. PACK

The dispersions of lipoids with the secession of dormancy in tissues may be of great significance and general physiological interest. The writer (3), while working on the physical and chemical changes involved in the awakening of dormant organs, found that the lipoids became dispersed as the tissues grew more active. It was further noted that the lipoids of storage and dormant cells were not so finely divided as those of actively growing cells. After a thorough rechecking of these results, the degree of lipoid dispersion was studied. Other tissues were examined to determine how general such dispersion was in the physiological processes involving fats.

To the writer's knowledge, little or no work has been directed along this particular line. This fact probably explains the conflicting statements that appear in the literature. As early as 1882, SACHS (5) stated that oil drops appear in the parenchyma of root and shoot axils of *Ricinus*. WAKKER (7) in 1888 reported small oil drops evenly distributed in the protoplasm of the youngest seed tissues. He also reported that oil appeared as equally large drops in all cells. In 1900 TSCHIRCH (6) stated that the oil of seeds was not in drop form, but homogeneously mixed with the cell plasm. Three years later ANDREW (1), while working on the separation of cell constituents by centrifugation, noted oil drops in *Ricinus* seeds. He also found that growth followed by centrifuging had a tendency to separate the oil into drops. CZAPEK (2) states that the fat of dry resting seeds is amicroscopically divided in the plasma, and that oil drops in the endosperm can at no time be demonstrated. He adds that oil drops become larger and fewer as germination proceeds.

Investigation

Tissues in different stages of dormancy and growth were sectioned with the freezing microtome, stained with Sudan III or Scharlach R, examined, cleared with glycerine, and studied further. The

glycerine increases the permanency of these preparations for continued study. This material was not mounted in water, but direct in the stain and examined. Other sections were stained with osmic acid. A third lot of tissue was fixed in strong chromo-osmic-acetic solution, washed, dehydrated, imbedded in paraffin, and sectioned. Some of these sections were mounted without staining, others were stained with alcannin, while others were stained with safranin, gentian violet, and orange G. The degree of lipoid dispersion was measured with the eyepiece micrometer. This was calibrated for each optic combination by the aid of the stage micrometer. Lipoid and oil are used here in their broad sense, indicating all fat staining materials

Results

Lipoid drops were demonstrated in the endosperm and embryo cells of the dry resting *Juniper* seeds. As these seeds slowly afterripened and became more active in a germinator at 5° C., the lipoid drops gradually became smaller and increased in number. This increased dispersion of the lipoids in germinated seeds over that of the lipoids in dry seeds was not due to histological treatment, since both the germinated and dry seeds received similar treatment. This was not only the case with the embryo cells, but also with the endosperm cells. The lipoid drops of the hypocotyl and outer embryo cells showed a great degree of dispersion and became very numerous. This dispersion was found to precede and accompany the appearance of sugar and starch in the hypocotyl region. Further chemical analysis (4) of these seeds showed that the larger part of the sugars and all the starch appeared after germination, while microscopic study of similar germinated seeds showed that the lipoids had become well dispersed. As the seeds germinated, the lipoid drops in the root and shoot axil reached a degree of dispersion equal to the limit of the microscope. During the germination period there was a decrease in the number of these lipoid drops, because this material was used in the manufacture of other compounds, as the chemical analysis shows (4).

The measurements of dispersion were made by several observers and the average taken. The largest oil drops noted in the dry seeds

were 20–30μ in diameter, while the smallest detectable oil drops of germinated seeds had a diameter of about 2 μ. There appeared in germinated seeds other probable oil drops that were of less and less diameters, which no doubt passed the limit of the microscope.

Other plant and animal tissues were examined to see how generally this dispersion accompanied active growth and digestion. Lipoid drops in the sunflower seeds were found to become dispersed as the seeds germinated. The seeds of hawthorn, castor bean, and peach were studied with the same results. It was also noted that many actively growing and dividing animal and plant cells contained no demonstrable fat. The highly dispersed condition of the lipoids in the intestines and blood of higher animals was noted.

Discussion

The physical state of lipoids seems to depend upon the particular tissue and the condition of the tissue. Resting seeds with large amounts of lipoid material often store it in the form of large drops. At times these drops are collected into masses, which fill the meshes of the protoplasm. If such tissue is stained with osmic and sectioned thickly, the whole cell is darkened and gives the impression that the fatty substance is evenly distributed throughout the cell. The massing of drops and bodies of lipoids may be distinguished in thin sections. In other instances individual drops occupy the major part of the cell cavity. Resting seeds with little fatty content deposit lipoids as fine drops or crystals. The lipoids of active cells and tissues are in a very fine state of division.

The fact that active cells and tissues contain exceedingly fine lipoid emulsions or lipoids that are undemonstrable by stains, necessitates a dispersion of this lipoid material at some time or other. The reasonable and actual time for this dispersion to occur is before the lipoids are decomposed, absorbed, translocated, or resynthesized. It would therefore accompany the secession of dormancy, the germination, or the activity of tissues in general.

A dispersion of the lipoids hastens these physiological processes. WENZEL's law states that the reaction velocity is proportional to the area of contact. Colloidal dispersion would increase the lipoid surface area hundreds of times, and accordingly the velocity of reac-

tion. In this way the hydrolysis of lipoids is hastened. Likewise the syntheses of various lipins, lecithoproteins, and other life requiring substances are favored. The syntheses of carbohydrates from fats is accordingly accelerated. If lipoid substances are hydrolyzed before absorption, or translocation, these processes are materially aided by lipoid dispersion; if lipoid substances are absorbed and translocated as such, they must of necessity be very highly dispersed. In either case dispersion should aid absorption and translocation. It seems that dispersion is of first importance, no matter what the following physiological changes may be.

Further physiological processes are accelerated by the action of catalysts or enzymes, whether acting chemically or physically. At the surface of all bodies there is a layer of molecules, which by their position are subjected to a new and different set of forces from those molecules in the interior of the liquid. They accordingly acquire a new set of conditions and functions. Then the colloidial particle has its absorption layer, which may be either a region of concentration or dilution. As a result, the lipoid enzymes would be concentrated in particular regions and thereby hasten digestion. This concentration of enzymes must be important when one considers the enormous amount of work required of a minimum quantity of enzyme.

The enzyme or catalytic power of seeds and tissues becomes more powerful as the seeds germinate or the tissue becomes active. This increased catalytic power may be related in some way to the dispersion of lipoids and other materials. It has been found that the maximum catalase activity and the maximum lipoid dispersion coincide. We should know if this is the case with the dispersion of other materials.

It may be of interest to recall the progress of events in the depositing of fatty substances in a storage organ. One first notices very fine oil droplets or bodies in the younger cells. These droplets increase in size with the age of the cells. In old cells large drops or masses of fatty substances can be demonstrated. These changes are the reverse of the dispersion of lipoids found to accompany the digestion, germination, etc., in active tissues. Just as condensation attends the storage of lipoids, so dispersion accompanies their digestion.

Acknowledgments are due to Dr. WILLIAM CROCKER, who followed this work with interest.

Summary

1. Lipoid drops were demonstrated in resting seeds.

2. The lipoids were found to disperse as the tissues became more active.

3. This dispersion reaches the limit of the microscope.

4. Lipoid dispersion is found in both plant and animal tissues.

5. A dispersion of the lipoids precedes lipoid decomposition, absorption, translocation, and synthesis.

6. These physiological processes are aided and hastened by lipoid dispersion.

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BRIEFER ARTICLES

EFFECT OF WOUNDING ON RESPIRATION AND EXCHANGE OF GASES

A number of references on the effect of wounding on respiration occur in the literature. PALLADIN[1] refers to one experiment in which 300 gm. of uninjured potato tubers produced from 1.2 to 2 mg. of carbon dioxide per hour. After the rate had been determined, each tuber was quartered, and the pieces were left at the same temperature and in the same surroundings as before. The rate of respiration increased up to the twenty-eighth hour after cutting, when it was 18.6 mg. per hour; then it began to decrease.

MAGNESS[2] has studied the effect of wounding on the internal atmosphere of apples. A thin slice of peel was removed from each end of the fruit, and they were then put in storage at the various temperatures, by the side of the whole fruit serving as checks. The data obtained showed that removing the epidermis greatly facilitates the entrance of gases to the tissues, and also the escape of accumulated carbon dioxide. MAGNESS remarks that it would be interesting to know to what extent increased respiration following wounding is due to mechanically facilitating this gaseous exchange, and to what extent it is due to actual metabolic changes in the wounded tissues.

Experiments by STICH[3] in 1891 showed that when 65.3 gm. of potato tubers were quartered, and bound together again and sealed, their rate of respiration increased from 4.3 mg. per hour before injury to 9.5 mg. after wounding; while another lot which was quartered but left free in the respiratory apparatus respired at the rate of 25.7 mg. per hour. These data indicate that 120.9 per cent of the increase in the rate of respiration is due to injury, while 497.6 minus 120.9, or 376.7 per cent of the increase in the later case, is due to facilitating the exchange of gases.

[1] PALLADIN, V. I., Plant physiology. 2d Eng. ed., edited by B. E. LIVINGSTON (p. 213).

[2] MAGNESS, J. R., Composition of gases in intercellular spaces of apples and potatoes. BOT. GAZ. **70**: 308–316. 1920.

[3] STICH, C., Die Athmung der Pflanzen bei Verminderter Saurspannung und bei Verletzungen. Flora **74**: 1–57. 1891.

Some experiments with wounded and uninjured sweet potatoes contribute further data on this problem. By means of a cork borer, cylindrical plugs were removed from one lot, producing an injured surface area of 7385.3 sq. mm. The rate of respiration increased from 64.27 mg. per kilogram hour before injury to 126.81 mg. per kilogram hour after injury. From a similar lot plugs were removed but replaced and sealed, producing an injured area of 7636.4 sq. mm. In this case the rate of respiration increased from 60.06 mg. per kilogram hour before injury to 70.36 mg. after wounding. Therefore the rate of respiration increased 97.3 per cent when the injured area was exposed, while it increased only 17.15 per cent when not exposed. It is evident that such increase is largely due to mechanically facilitating the exchange of gases, rather than to direct wound stimulation.—G. R. JOHNSTONE, *University of Southern California, Los Angeles, Cal.*

CURRENT LITERATURE

NOTES FOR STUDENTS

Self-sterility.—The bulk of investigation of this problem has been about as sterile as the material used. From the viewpoint of genetics, however, there is one set of experiments that has repeatedly borne fruit of fascinating quality. These are the experiments upon *Nicotiana*, which have been continued for a number of years by EAST and his associates.[1] The gross physiology of self-sterility has clearly been shown to be a matter of pollen tube growth. Own pollen tubes exhibit a slow and steady rate of growth which ordinarily does not permit fertilization to occur within the life of the flower; while foreign pollen tubes grow at such a rapidly accelerated rate as to bring successful fertilization.

The basic genetical problem of self-sterility was rather readily solved, for self-sterility was found to be a Mendelian recessive in crosses between self-sterile and self-fertile species. A secondary problem was presented by the relation of self-sterile plants inter se, and this proved to be considerably more involved. In the earlier experiments the self-sterile plants were found to be completely cross-fertile; that is, every self-sterile individual was fertile in crosses with every other self-sterile individual. This was accounted for by assuming that every one of these individuals differed somewhat from every other, and this difference brought cross-fertility. Presumably then, two individuals of identical genetical constitution would prove cross-sterile. To test this hypothesis the attempt was made to obtain groups of individuals of the same genotype. This, of course, could be accomplished if self-sterile individuals could be

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EAST, E. M., and MANGELSDORF, A. J., A new interpretation of the hereditary behavior of self-sterile plants. Proc. Nat. Acad. Sci. **11**:166–171. 1925.

inbred, an act which would seem by definition to be impossible. Since self-sterility, however, was merely the result of the slow growth of own pollen tubes, one might hope to bring about self-fertilization by giving the own pollen tubes an abnormally early start. As a matter of fact, pollination in the very young bud did bring the desired result; self-sterile individuals were thus inbred. The progeny so obtained (in a few generations) fell into several groups, such that every individual of a group was cross-sterile with every other individual of that same group, although cross-fertile with individuals of other groups. Thus cross-fertility was shown to be due to genetic difference.

Following this, the interrelationship of these various sterility groups was examined critically, and a surprising genetic phenomenon brought to light. For example, in a family composed of three such intra-sterile groups, X, Y, and Z, the following breeding results were obtained. X female mated with Y male gives, in equal numbers, progeny of types Y and Z, but none of X. Y female with X male gives progeny of X and Z, but none of Y. Similar results are obtained from reciprocal crosses of Y and Z and X and Z. In short, two classes always appear in equal numbers, but the class of the mother is never represented.

These surprising results, taken together with certain others which are more involved but increasingly convincing, support the following interesting genetical interpretation. The sterility phenomena are governed by a series of three multiple allelomorphs, S_1, S_2, and S_3. Sterility group X is of constitution S_1S_3, Y is S_1S_2, and Z is S_2S_3. The X female, S_1S_3, stimulates only those tubes containing the allelomorph she lacks. Mated with a Y male, she would be presented with pollen tubes carrying S_1 and others carrying S_2. Only the latter are successful; hence the only progeny produced are S_1S_2(Y) and S_2S_3(Z). Comparable results are produced in the other possible reciprocal crosses.—M. C. COULTER.

Taxonomic notes.—CHODAT,[2] in continuation of his studies of the alpine flora of Switzerland (Grand St. Bernard), has published an account of 7 genera, including a new genus (*Bernardium*)and new species in *Anabaena* and *Glenodinium*.

YAMAMOTO[3] has published a new genus of Orchidaceae from Formosa, under the descriptive name *Tuberolabium*.

HONDA,[4] in his revision of the grasses of Japan, appearing in a series of contributions, has now included 83 species. In the last four contributions 54 species are presented, including a new genus (*Eulaliopsis*), 7 new species, and many new varieties.

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[2] CHODAT, R., Algues de la région du Grand Saint-Bernard. Bull. Soc. Bot. Genève. pp. 33–48. 1924.

[3] YAMAMOTO, Y., Genus novum Orchidacearum ex Formosa. Bot. Mag. Tokyo **38**: 209–212. 1924.

[4] HONDA, M., Revisio Graminum Japoniae. IV–VII. Bot. Mag. Tokyo **38**: 49–59, 119–129, 189–201. 1924; **39**: 33–43. 1925.

KOIDZUMI,[5] in his investigation of the flora of Oriental Asia, has published 61 new species, including a new genus of Liliaceae (*Brachycirtis*) and of Gramineae (*Chikusichloa*).

BRAINERD[6] has published a full and well illustrated account of 82 natural violet hybrids. The plants studied have been secured from all parts of the United States and some provinces in Canada, transplanted at Middlebury, Vermont, and kept under experimental control. The results and conclusions are of great interest in connection with the problems of inheritance and limitations of species.

MCNAIR[7] has investigated the taxonomy of "poison ivy" in great detail, and has reached the conclusion that many of the new species proposed during recent years are synonymous. He recognizes only four species of *Rhus* included in the poison ivy group, and has described them in detail, with full synonymy and collections. This certainly reduces a very long list of specific names to synonymns. The species recognized are *R. quercifolia*, *R. diversiloba*, *R. Toxicodendron*, and *R. divaricata*.

NANNFELDT[8] has revised a section (Asiaticae) of *Centella*, a tropical genus of Umbelliferae, recognizing 11 species, 3 of which are described as new, and 4 transferred.

FITZPATRICK[9] has made a critical study of the Pyrenomycete genus *Fracchiaea* occurring on branches of many trees, recognizing 16 species.

ST. JOHN and PARKER[10] have described a new subgenus (*Altericarex*) of *Carex*, distinguished by having four stigmas and a 4-angled achene, which includes the new section Tetragonae, containing one species (*C. concinnoides*) of western North America.—J. M. C.

Evaporation and transpiration.—A few years ago BATES[11] devised a metal atmometer for use in forest studies that resembled somewhat a foliage leaf in its structure. It had an impervious upper surface and a perforated lower surface with porous moist material placed between. Experiments showed that the losses

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[5] KOIDZUMI, G., Contributiones ad cognitionem florae Asiae Orientalis. Bot. Mag. Tokyo **38**:87–113. 1924; **39**:1–30. 1925.

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from this instrument followed the transpirational losses from small coniferous trees rather closely. The inventor made no further claims that it would measure the transpiration from these or other plants. He evidently intended that it should be used as an instrument that would give some approximate determination of the environmental conditions that affect the rate of water loss by vegetation; in other words, it would measure what has been called the "evaporating power of the air."

A different understanding has led SINGH[12] to make some comparisons between rates of evaporation from a shallow open pan and from Bates atmometer and the transpiration losses from a jasmine plant. He obtained results very similar to those formerly obtained by SHANTZ for alfalfa. He naturally reaches the conclusion, already held by practically all ecologists, that there are regulatory factors that affect water losses from plants that do not influence the losses from atmometers or evaporimeters.

Another recent study by DOLE[13] of some of the conditions that determine variations in transpiration and evaporation has shown that the previous environmental history of the plant affects its rate of water loss, as does the amount of available soil water, indicating that in critical studies of transpiration a controlled environment is imperative. He further shows that in an unchanging controlled environment the phenomena of transpiration and evaporation are closely parallel. This he takes to mean that deviations from the same tendencies in the two phenomena in a varying environment are due chiefly to the secondary effects of the environment upon the organism, and to the changes within the organism thus induced. DOLE's report is to be commended for its care in the analysis and control of environment, and for the carefully considered conclusions.—GEO. D. FULLER.

Illinois State Academy of Science.—The *Transactions* for the 1924 meeting (vol. 17. pp. 444) include the following papers of botanical interest: Plant communities of Glacier National Park, W. G. WATERMAN; A new mushroom (*Marrasmius nucicola*), W. B. MCDOUGALL; Presence of living organisms in lake ice, S. EDDY; Some outstanding features of the plant disease situation in Illinois during 1923, L. R. TEHON; Some north and south stream valleys in Illinois and their vegetation, G. D. FULLER and C. J. TELFORD; Preliminary check list of the vascular plants of the Illinois State Park at Starved Rock, F. THONE; Forest preservation, E. M. NORTH; The cotton industry of Southern Illinois, F. H. COLYER.—J. M. C.

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